AUGUST 2015 # 10

the Pathologist

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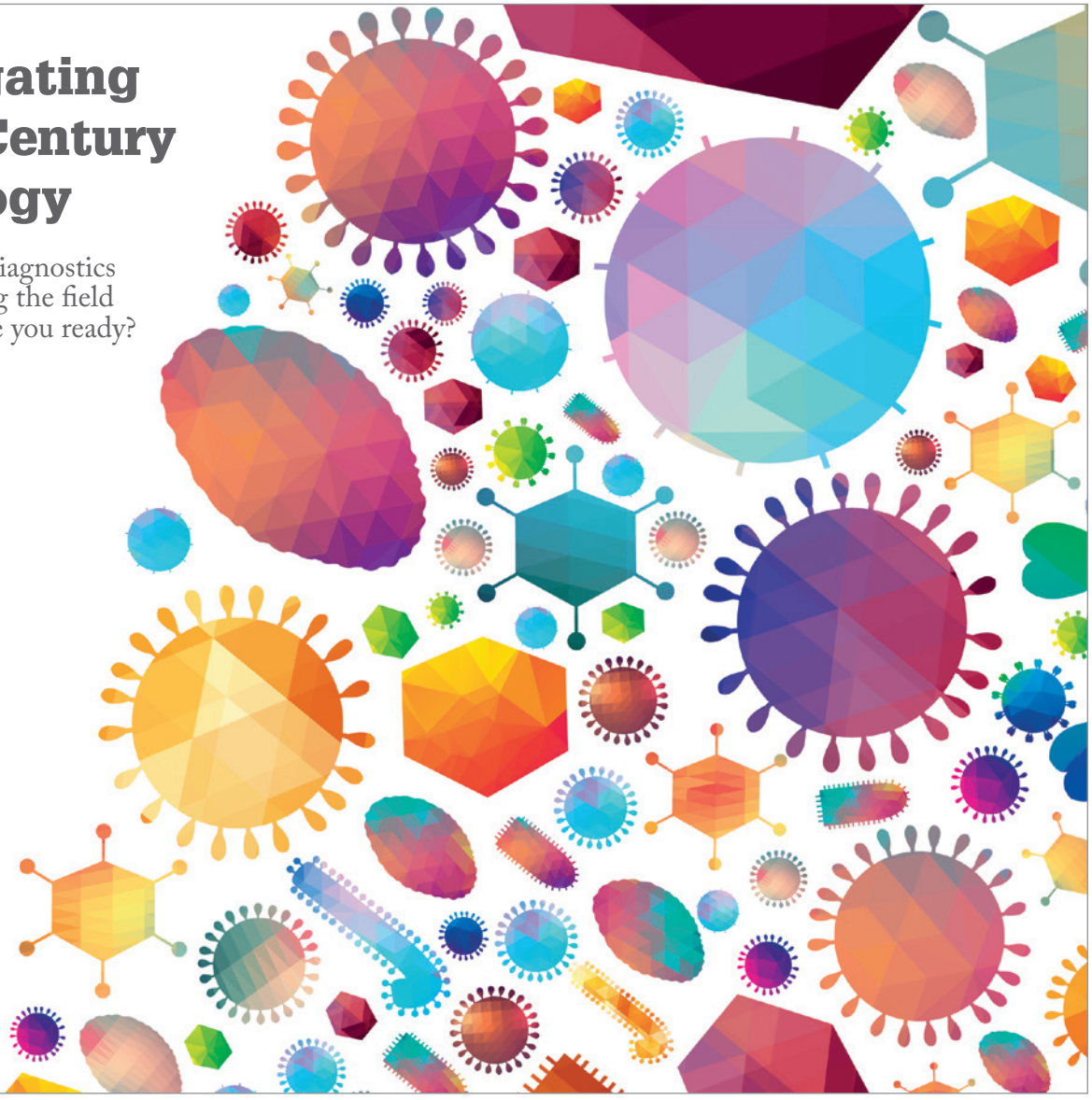

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5 - 9 September, 2015

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Online this Month

The Pathologist Power List 2015 – We Need Your Vote!

- ✓ Do you want your area of lab medicine to gain more publicity?
- ✓ Do you feel the contributions of those you admire are going unnoticed?
- ✓ Do you think your profession is unfairly stereotyped?
- ✓ Do you want to shine a light on role models that are inspiring change in lab medicine?

If you answer "yes" to any of the above, now is your chance to have your voice heard.

Nominate your lab medicine champion today at:
http://tp.txp.to/powerlist_form

Make sure that all of laboratory medicine's unsung heroes are recognized for their achievements.

Nominees will be judged by a panel of experts and the top 100 will be published in the November issue of The Pathologist.

The Power List is celebrating success in all fields of laboratory medicine.

European Congress of Pathology, Belgrade, 5–9 September, 2015

ESP President Han van Krieken offers his top tips for this year's event

1. Keynote lectures

"For me, the keynote lectures are always the highlight. This year's lecturers are four excellent speakers who look at pathology from a slightly different angle than the usual. There will be a very interesting lecture on how the immune system affects cancer, how that's now entering the clinic, and what aspects of it we can see in our tissues. There will also be a talk on viruses and how the role of the pathologist figures into addressing viral pathogens. Furthermore we will get insight in the new approach for WHO-classification and on forms of microscopical imaging."

2. Special sessions

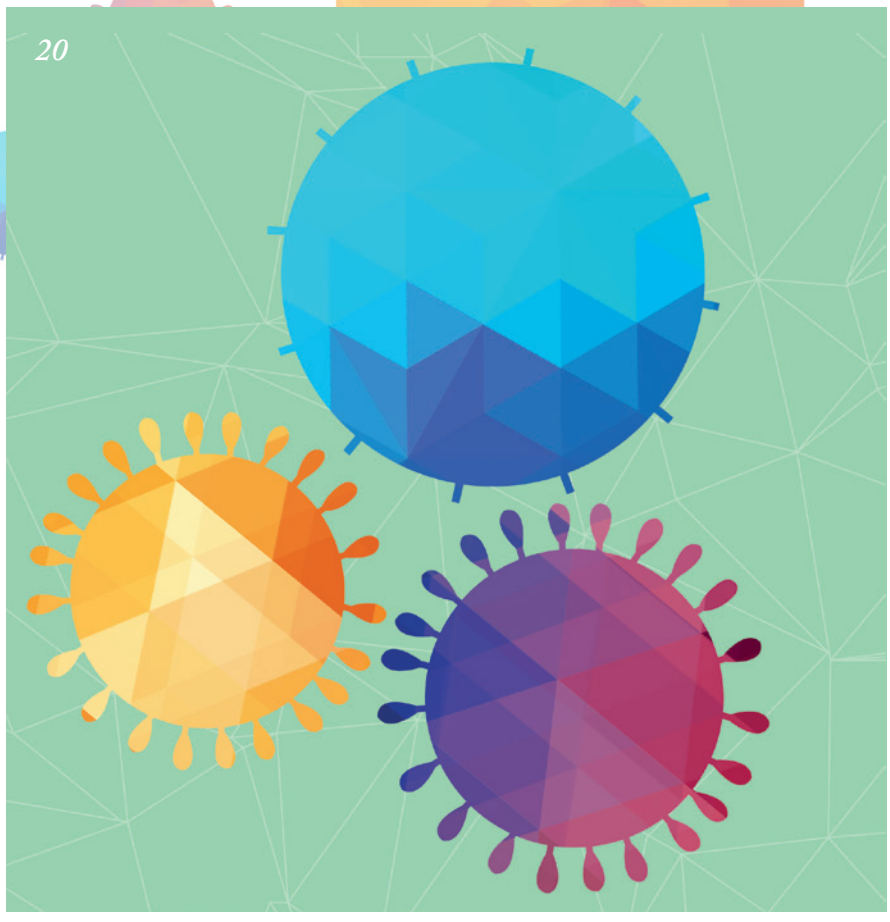
"We have a few special sessions this year. One will be a workshop on pathology and the public – we would like to encourage pathologists to engage members of the public more. Another is a two-day session fully dedicated to molecular pathology for molecular biologists, with a focus on bringing molecular biologists in pathology together and it's also interesting for clinical pathologists who want to have in-depth knowledge of molecular pathology. It's the first time we will have such a program."

3. New to molecular?

"We've also integrated molecular pathology into many of the programs that appeal to clinical pathologists. In the sessions on lung and colon cancer, for instance, there will be a lot of practical molecular biology. That's what I'd recommend for pathologists who are still new to molecular pathology."

Go to http://bit.ly/1MtaFvv to view the full scientific program.

Contents



On The Cover

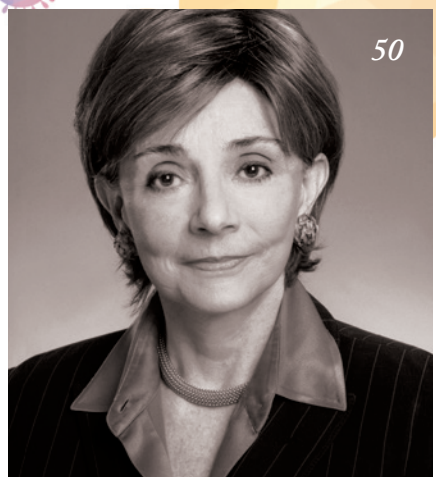
A graphical representation of the unique and diverse nature of viruses.

Upfront

In My View

Feature

36



50

says Jan Barghaan.

In Practice

NextGen

Profession

Sitting Down With

the Pathologist

ISSUE 10 - AUGUST 2015

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You've Got the Power!

Let's celebrate the successes of our field by shining a spotlight on the people who drive it forward.

The Pathologist is on a mission to bolster the profile of laboratory medicine. To that end, we are launching a brave initiative: The Power List – a celebration of the top 100 people in laboratory medicine. Your mission? To nominate the individuals who have inspired you – whatever your specialty.

Why are we doing this? We communicate with people in laboratory medicine every day – we learn of your challenges, we gauge the potential impact of exciting new techniques based on your opinions, and we discover how policies are likely to affect your working life. But the message that comes through strongest of all are your concerns about the low profile of laboratory medicine. This lack of prestige not only makes it difficult to attract new talent, but also raises barriers to the funding you so desperately need to keep pace with a rapidly changing environment. Fortunately, we also hear your solutions loud and clear: "We must communicate more," "We have to alter the perception," "We need to collaborate with others and educate them," "We need to do more if our profession is going to survive"...

Educating other medical professions, governments and the public on the value of your role is no easy task. We believe that by very publically acknowledging the pioneers and unassuming legends of the laboratory world, from microbiologists to microscopists, from clinical biochemists to molecular biologists – and everyone in between – The Power List can have a positive impact. In short, we want to celebrate the diversity and importance of our field by shouting about the people who are making a real difference.

Clearly, only the lab medicine community itself can help compile such a list, so we need you to get involved by nominating the individuals who deserve kudos. How? Simply fill out our short online form: http://tp.txp.to/powerlist_form. All nominations will be counted and then reviewed by an independent panel of expert judges who will make the final selection of 100 people for The Pathologist's 2015 Power List.

To those who think this is a futile exercise: think again! Our pledge is to disseminate The Power List to as wide an audience as possible, and we have no doubt that the outcome will surprise you (in a good way). Let's use The Power List to highlight the pioneering achievements of those who are shaping laboratory medicine and contributing to real improvements in patient care. Together, we can make your voices heard and celebrate all of our successes.

Cast your nomination for The Power List today at http://tp.txp.to/powerlist_form.

Fedra Pavlou
Editor

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: fedra.pavlou@texerepublishing.com

New York Severs Pathologist-Patient Connection

Pathologists and hospitals urge New York State to revoke a bill that bans direct interaction of pathologists with patients

The New York City Health and Hospitals Corporation, which operates the public hospitals and clinics within the city, have joined New York pathologists and CAP (the College of American Pathologists) in a call to repeal a bill previously passed by the New York State Senate (1). The bill in question allows patients to receive results direct from the lab upon request, but prevents the pathologist who performed the test (and understands the results) from interacting with the recipients and explaining the meaning of said test. Critics of the bill claim that this could leave already anxious patients in a worse state when presented with a sheet of paper with figures and alarming science which they may not understand. "Given the advances in diagnostics and explosion in diagnostic data, pathologists interacting with patients directly as part of the healthcare team can only enhance patient outcomes and patient satisfaction," says Michael Prystowsky, professor and university chairman of pathology at Montefiore Medical Center and Albert Einstein College of Medicine, New York.

The bill, which ironically also blocked any laws preventing patients directly receiving test results from the lab, was finalized in February 2014 despite protests from CAP, which felt that results were best explained thoroughly by a physician when first presented to the patient in order to eliminate confusion and to address patients' concerns (2). The state declined the repeal, however, and responded by stating, "A clinical laboratory that provides a patient with the meaning or interpretation of the test results is discharging the ordering physician's responsibilities and such discharge is of decided benefit to the physician, precluding the practitioner from incurring expenses or expending time," (3).

This comes at a time when pathologists are being increasingly encouraged to become more and more involved in patient care. And rightly so; the benefits for the patient, clinician and pathologist are obvious, but this interaction is especially important in those cases where clinicians are not actually confident in interpreting the results of a test. Worryingly, research has found this to be true in some cases; for example, in a survey of UK junior doctors, over 40 percent admitted that they were not confident in interpreting results for laboratory tests such as urine sodium and osmolality tests (4). Furthermore, when Gerd Gigerenzer of the Max Planck Institute for Human Development in Berlin, asked 160 gynecologists how many women testing positive on mammogram screening actually have breast cancer, disconcertingly, the majority answered 81 or 90 percent. Only 21 percent correctly answered one in 10 (5). These are just two of many studies that add credit to the pathologist-patient link and fuel the argument against the bill.

New York pathologists have been quick to show their disagreement to the new rules; physicians located throughout New York have affixed their signatures to a CAP response providing additional arguments and references for the repeal (6). "As physicians, pathologists have a legal and ethical obligation to care for their patients," says Prystowsky. The pathology community is not going to take this lying down. But will New York listen? *JR*

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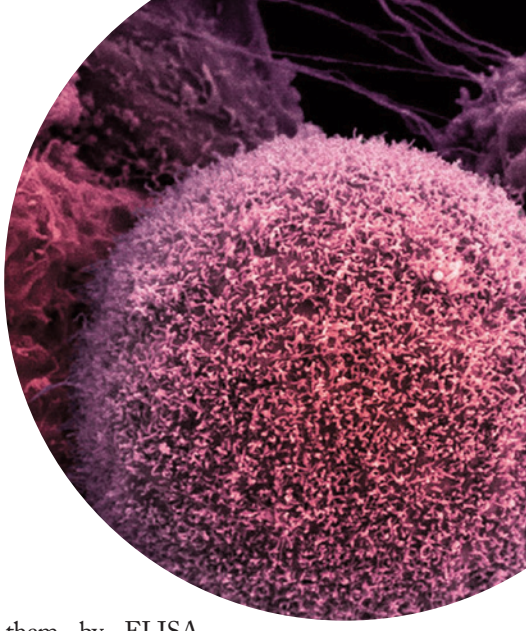
High Hopes for Pancreatic Cancer Urine Test

A new three-biomarker panel may allow early detection of the disease at a stage when curative surgical treatment may still be possible

Cancer mortality is going down across the board as patients with a wide variety of cancers experience earlier diagnosis and longer overall survival (1). But not every type of disease shares in the good news. Pancreatic cancer still has one of the lowest survival rates of any cancer, with a median survival of six months or less – and almost no improvement over the last three decades. As with most cancers, the main obstacle to successful treatment is timely diagnosis; although five-year survival rates for patients with incidental diagnosis and resection of early-stage tumors can reach 60 percent (2), very few people fit into this category. The reason: lack of symptoms in the early stages of disease and poor diagnostic modalities – meaning most pancreatic cancer cases are diagnosed after metastasis has occurred (3).

One group of scientists from Queen Mary University of London is hoping to change this poor outlook. They have identified three biomarkers in urine that may signal the presence of pancreatic cancer before symptoms even emerge (4). Could this mean that physicians might soon be able to identify patients with early-stage disease inexpensively and noninvasively? That's the hope. The research team base their optimism on the findings of an in-depth proteomics analysis of 18 urine samples (three male and three female, each with pancreatic ductal adenocarcinoma, chronic pancreatitis or no disease), which revealed that three specific proteins – LYVE-1 (a marker of lymphatic endothelial cells), TFF1 (a mucosal protein), and REG1A (a member of the regenerating protein family secreted by the exocrine pancreas) – were deregulated in both males and females with pancreatic cancer. Scaling up to 371 urine samples (from London, Liverpool and Madrid) added further weight to the evidence: all pancreatic cancer patients showed higher urine concentrations of the candidate biomarkers. Best of all, the elevated biomarkers were not only present in the later stages of disease, but even in patients with stage I disease. This provides hope that such a test could distinguish patients with early-stage pancreatic ductal adenocarcinoma from healthy individuals or, potentially, those with other hepatobiliary disease.

The study is a strong one – after identifying the biomarkers by mass spectrometry, the researchers validated



them by ELISA and examined them in 488 urine samples from three separate healthcare centers. But the work isn't finished yet; the biomarkers need further validation. The study's authors point out that their control population was younger than their cancer patient population, and suggest conducting a similar study with older controls. They also suggest testing the biomarker panel on high-risk groups, collecting more long-term data, and further comparing their biomarkers to the known pancreatic tumor marker CA19-9, which when combined with the new biomarker panel, may increase accuracy. So there's a lot still to be done before clinical testing with this panel becomes a reality – but identifying and validating the biomarkers is still a significant first step. *MS*

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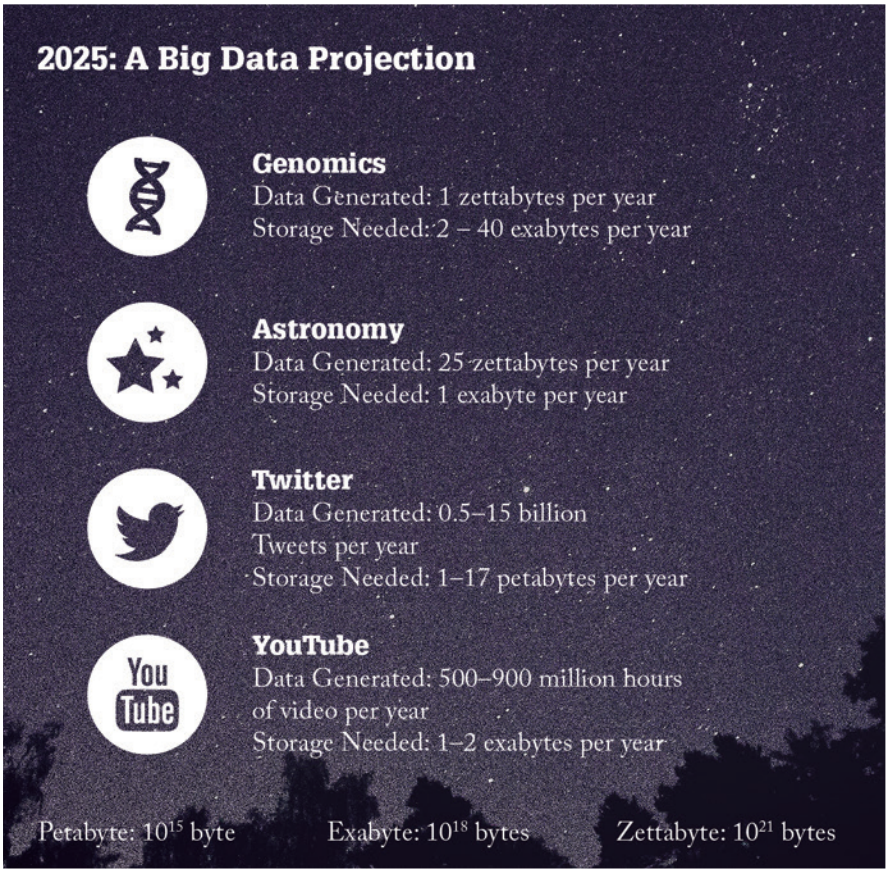

Forget Astronomical, Try Genomical

Genomics could soon outpace other scientific disciplines as the king of big data, but there could be problems ahead…

The genomics revolution is uncovering more insights into human biology than ever before, but some researchers foresee a problem on the horizon: just what will we do with all that information? A recent study by a team of US biologists and data scientists has concluded that the speed of genomic data generation has now outstripped that of YouTube (1), and at the current rate, the amount of genomic data produced every day is doubling every seven months.

Right now the storage and analysis of genomic information is manageable, but as sequencing becomes cheaper and more common, issues are likely to arise. It's predicted that by 2025, up to a billion people may have had their genomes sequenced, creating the need for a huge amount of storage, and producing vast amounts of data on par with social media platforms, and disciplines such as astronomy (see Figure).

Genomics is a "four-headed beast", explain the researchers, with four key areas: acquisition, storage, distribution and analysis; all posing their own particular challenges. This means that no one solution will solve the impending problem – improved sequencing technologies, data storage and sharing solutions, and optimized computing infrastructures and data libraries will all need to play a part as genomics grows at lightning speed. "For a very long time, people have used the adjective 'astronomical' to talk about things that are really, truly huge," says Michael Schatz, co-author of the associated paper, "but in pointing out the incredible pace of growth of data-generation in the biological sciences, my colleagues and I are suggesting we may need to start calling truly immense things 'genomical' in the years just ahead." *RM*

Reference

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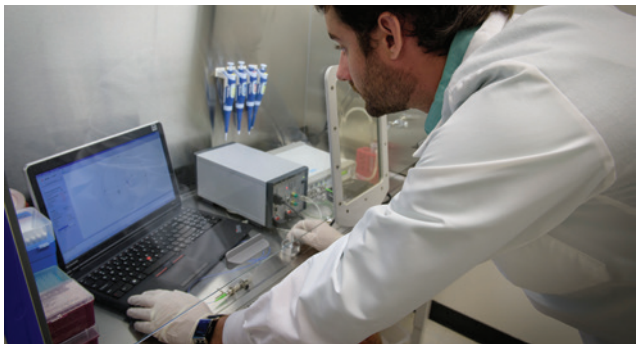
Squishy Science

Could a tumor "squishiness" detector open a new avenue of cancer research?

The exciting field of molecular diagnostics is yielding some game-changing discoveries in oncology, providing hope for early intervention and increased survival rates. But are molecular and genetic biomarker assays truly the best approach for monitoring cancer progression? Conventional wisdom says yes, but innovation rarely follows convention. Results emerging from the NIH's Physical Science Oncology Centers (PSOC) are showing the possibility of a new class of biomarkers based not on a tumor's chemical properties, but on its physical properties. However, unlike with chemical biomarker assays that can be performed in a high throughput manner, screening for hundreds or even thousands of biomarkers at once, performing these mechanical measurements is a painstakingly slow



process. "Oncologists and physicians might view this as a barrier, but as engineers, we saw it as an exciting challenge," says Andrea Armani, part of the team who decided to tackle the problem.

"Dr. David Agus of the University of Southern California's Keck Medical School was intrigued by recent studies which showed tumors to have very different Young's moduli (YM) as compared with healthy tissue. But he was frustrated at the complex and slow methods used to measure this value, so he tasked my group with finding a simple and quick method to perform this measurement", recalls Armani.

The Young's modulus describes the amount of force needed to compress a sample, and is a measurement of elasticity (or "squishiness"). Instruments to measure YM already exist, but are cumbersome, sensitive to environmental vibrations, and require recalibration by a trained user when moved. Armani and her colleagues solved the problem with optical fiber: the sample is compressed on top of an optical fiber, which changes the polarization of the light inside, allowing the YM to be calculated (1). The new instrument is the size of a back pack, easy to use, and is fully portable.

"It is important to recognize that the fundamental field of correlating mechanical markers (such as elasticity) to cancer prognosis is relatively new. Our method could prove to be extremely useful in these studies – and because it is non-destructive, samples can be subsequently tested using molecular or cellular methods, such as genomic profiling," says Armani.

While initial results have shown that more aggressive tumors seem to be stiffer, more research is needed. "This advancement from Dr. Armani is so exciting, as we now have a new dimension of tumor to measure," adds David Agus. *RM*

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Diabetes Detector

A new blood test could detect type 1 diabetes decades before symptoms develop

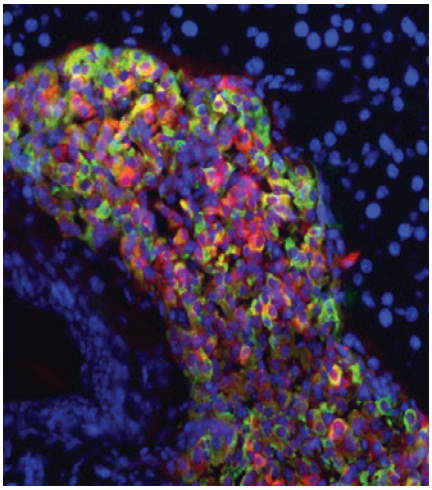
Diagnosing type 1 diabetes is problematic. Patients only seek out help once symptoms start to arise, but the early stages of the autoimmune disease have already been at work for a number of years. Up until now, there has been no reliable method of detecting early stage, presymptomatic diabetes; however, a recent discovery could be set to change all of that. Scientists at the Medical Research Council's Clinical Science Centre (CSC) in London, in collaboration with scientists at the Swiss Federal Institute of Technology, have identified microRNAs (miRNAs) that could act as effective biomarkers years before symptoms even develop, as they circulate in the blood during the early stages if the disease (1).

Specifically, the research team honed in on one miRNA – miR-375. No stranger to being used as a biomarker (having previously been cited (2) as a potential indicator of prostate cancer), studies have revealed miR-375 to be the most abundant among the many miRNAs found within β-cells (3). In fact, mice unable to produce the molecular messenger have shown a decrease in β-cell mass and subsequently develop diabetes, suggesting a crucial role for miR-375 in blood sugar regulation (3). It was this research that inspired Mathieu Latreille, lead of CSC's Cellular Identity and Metabolism research group, to delve deeper. He explains, "Interestingly, some miRNA molecules have been shown to circulate in body fluids such as the blood, saliva, breast milk and other secretions and have recently become novel markers for several diseases. These findings motivated us to determine if miR-375 could be used as a marker to diagnose the destruction of β-cells that underlies the development of diabetes."

By analyzing the plasma of β-cell rescue mouse models, Latreille and his team found that a small but significant proportion of circulating miRNA is derived from pancreatic β-cells; his team is the first to demonstrate that β-cells release miR-375 into blood circulation. With these results under his belt, Latreille hypothesized that when these cells become targets of the immune system, the molecular messenger is released into the bloodstream at high quantities as the cells die.

Different models of β-cell stress were created by Latreille to test this hypothesis, who used chemical agents and genetic mutations to damage the insulin-producing cells. One such experiment involved treating mice with streptozotocin (STZ), a molecule which specifically kills β-cells. Three days after STZ injection, the team observed the expected rise in blood glucose as a result of β-cell destruction, but also measured a two-fold increase in miR-375 when compared with controls. Latreille explains that considering the contribution of β-cells to miR-375 levels in the blood is small, he believes that the most likely explanation for this observation is that hyperglycemia *per se* elicits increased miR-375 secretion from tissues other than pancreatic β-cells. This result encouraged the team to conduct further studies in humans, which complimented these findings when type 1 diabetes patients also showed elevated levels of miR-375.

Whilst the study showed no significant changes in miRNA circulation in type 2 diabetes patients, Latreille believes that in the future, a simple blood test could use miR-375 as an indicator of acute β-cell destruction and autoimmune diabetes. "If we can identify and treat patients earlier, we may be able to help them to avoid secondary complications. This could ultimately extend a patient's life," says Latreille. The team now intends to determine whether miR-375 could predict, with greater efficiency than current tests, if an individual is developing type 1 diabetes, years before symptoms arise, and if the miRNA could be effectively used as a surrogate biomarker for the disease. *JR*



The islets of Langerhans in a patient with type 1 diabetes after receiving treatment to transform α-cells into new β-cells (shown in green). Image courtesy of Monica Courtney, University of Nice-Sophia Antipolis, France.

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Smart Mouth

Alzheimer's disease predictive biomarkers in saliva may yield a simple, noninvasive diagnostic test

With the baby boomer population aging and incidence of Alzheimer's disease on the rise, it seems that everyone is racing to find a definitive diagnostic test for the condition. Currently, Alzheimer's is diagnosed by a series of cognitive tests, with biological confirmation possible only by postmortem examination of the brain. But cognitive tests aren't always reliable, and postmortem examinations have no impact on patient care – how can doctors ensure that they're diagnosing Alzheimer's as early and as effectively as possible?

New research from the University of Alberta, Canada suggests that the answer may lie in saliva. At the recent Alzheimer's Association International Conference, neuroscience student Shraddha Sapkota reported that metabolomic analysis of salivary samples by liquid chromatography-mass spectrometry allows clear discrimination between patients with Alzheimer's, those with mild cognitive impairment, and those with normal cognitive aging (1). The researchers were also able to identify the top metabolites for distinguishing between conditions. Most importantly, the study results revealed directional associations between certain metabolites and cognition states – in other words, biomarkers upregulated in patients with known cognitive impairments were predictive of episodic memory problems and slow neurocognition when elevated in those with normal aging. Detecting such biomarkers in saliva tests may eventually allow doctors to identify Alzheimer's patients before they become symptomatic – allowing critical early interventions to slow or even halt the progression of the disease. Not only that, but gaining this knowledge before cognitive issues become evident allows patients to take part in decision-making processes while still able, and allows researchers to identify potential clinical trial participants for Alzheimer's disease therapeutics or preventatives.

The benefits of the research extend beyond identifying predictive biomarkers. The saliva test itself is a new approach to Alzheimer's diagnosis and has advantages of its own. Saliva testing is easy, painless and noninvasive, and the fluid itself is easy to transport and already commonly used to test for many conditions. Especially in a scenario such as cognitive decline, which might require repeated testing over a long time span, it's an ideal choice. So how close are we to a widely available saliva test for Alzheimer's disease? The work is still in its early stages and requires much more research – but for the time being, salivary biomarkers show clear diagnostic promise. *MS*

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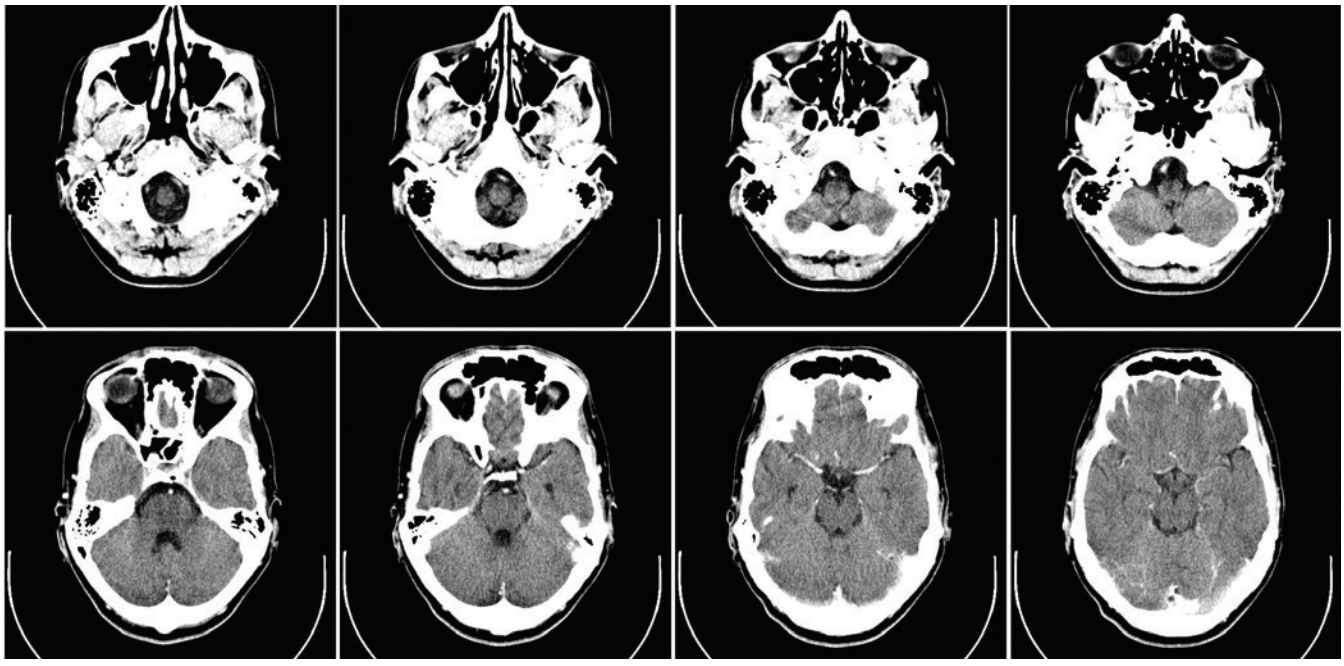

Brain Injury Blood Test Breakthrough?

A lab test for CNS-specific proteins could cut the number of unnecessary brain scans

An international study claims that a simple blood test can accurately predict the presence and the severity of traumatic brain injury (TBI). Not only would the availability of such a test in the clinical lab reduce the costs of unnecessary radiological examinations, but it would also allow a quicker patient categorization and provide valuable support for treatment decision-making.

The rationale behind the test is this: glial fibrillary acidic protein breakdown products (GFAP-BDP) are proteins found in the central nervous system (CNS), which can be detected in the serum using sandwich ELISA. These proteins are known to be associated with certain neurological disorders, including TBI. So an international team, headed by Paul McMahon of the University of Pittsburgh Medical Center, US, set about validating the use of this test in the diagnosis of intracranial injury in a broad population of patients with a positive clinical screen for head injury.

To do this, blood samples were analyzed in multiple centers, in over 200 patients aged 16–93 who were being treated for suspected TBI (1). Blood was drawn and tested for the GFAP-BDP biomarker within 24 hours of the patients presenting at a clinic, alongside CT scans. Patients were also offered a follow-up MRI within two weeks of the original injury.

The results were encouraging: elevated GFAP-BDP was significantly associated with the presence of visible TBI on CT scans, and the severity of injury. The test provided an advantage over clinical screening alone, preventing unnecessary scans by 12–30 percent, and predicted brain pathology on CT scan with an accuracy of 81 percent, higher than that of standard clinical predictors, such as pupillary response and Glasgow Coma Scale score.

Radiography is a central part of diagnosing brain injury, but scans can be expensive, and pose risks to the patient. The study authors are hopeful the test could become a useful addition to methods of neurological examination, and believe that "early measurement of GFAP-BDP can contribute to more accurate diagnosis and triage of TBI patients, decreasing the number of unnecessary CT scans and allowing more tailored management of the brain injury." *RM*

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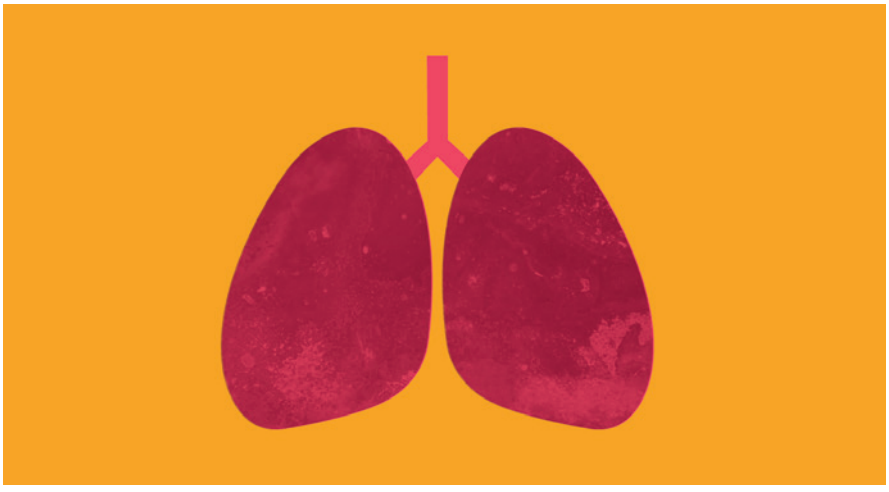
The Search for Next Gen Lung Cancer Diagnostics

Research discoveries continue to improve the outlook, but how promising are they?



Early-stage lung cancer is a diagnostic challenge; symptoms often don't develop until the disease is advanced, and widespread screening is not currently an option. Since faster detection is key to improved survival rates, finding better ways to catch and categorize lung cancer would be a boon to oncology. Unsurprisingly, the search for diagnostics is a hotbed of research and here we report on two teams who are shedding new light.

The first, a team headed up by Gerard Silvestri of the Medical University of South Carolina, USA, has been on the search for a diagnostic method that can be used alongside bronchoscopy to improve diagnostic rate. Their potential solution: a genomic classifier. "We needed better tools to predict whether lung lesions detected on X-ray or CT scan were cancer. Our minimally invasive procedures were not always giving us answers, requiring us to perform invasive surgery, and for some patients this turned out to be unnecessary," explains Silvestri.

A genomic classifier based on the expression of 23 genes and patient age was tested on respiratory epithelial cells collected from current and former smokers undergoing bronchoscopy for suspected lung cancer. The rationale for using the classifier was firmly justified when Silvestri and his colleagues found it to improve the rate of detection from 75 to an impressive 97 percent (1). Speaking of its potential applications in the clinic, "In patients with moderate risk of cancer, a non-diagnostic bronchoscopy and a negative genomic classifier would provide a safe means of serial imaging, rather than moving to surgery," says Silvestri.

For cases where biopsy does becomes necessary though, a second team from Ruhr University Bochum, Germany, has developed a fast, non-destructive and automated system for classifying cancerous tissue. The technique, named spectral histopathology (SHP), uses vibration spectra on lung biopsy sections to identify cancerous changes.

Because it relies on computational analysis of the spectra, no dyes are required, and tissue can be reused for H&E staining or molecular testing. A reference database, compiled with the input of pathologists, is used to automatically allocate the spectra to the matching tissue type, helping to classify tumors and predict disease aggressiveness. So far, SHP can identify all tumor classes, and distinguish prognostic subtypes of adenocarcinomas.

How effective is it? A study of 101 patient samples classified using SHP showed 97 percent accuracy when classifying tumor type, and 95 percent accuracy in subclassifying adenocarcinoma (2). The researchers now hope to further validate and develop the project with a larger, independent study.

What does this mean for pathologists? SHP has several potential, relevant applications – spectra analysis could be used alongside traditional staining to give pathologists a "second opinion", providing additional data when classifying cancers, which could help improve prognostic accuracy. It could also see use in the operating theatre – as the method employs light beams, the system could even be used on live tissue, allowing for fast analysis during surgery.

Both of these techniques require further validation before they hit the clinic, but it's clear that the hunt for ways to make lung cancer diagnosis faster, more accurate, and less invasive for patients is yielding some very promising results. *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.

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Revisiting Posthumous Analysis

It's time to take another look at the alternative to conventional autopsy methods

By Juan Rosai

Fedra Pavlou's editorial ("Autopsy on the Slab?") in the June 2015 issue of The Pathologist, highlighted that not much has changed in the 20 years since publishing my own article on this emotive subject (1). Historically, autopsies were extremely important as they opened up a new world of understanding of disease. As Bichat said, "You can take notes for 25 years, from morning to evening by the patients' bedsides on diseases of the lung, heart, and stomach and the result will be a long list of confusing symptoms leading to incoherent conclusions. Open a few bodies and you will see darkness immediately recede."

Indeed, in the early years of the 20th century, autopsies proved to be the best way to obtain important information that was impossible to glean in any other way. And, that was why clinicians requested them as a matter of course. In those days, academic institutions were performing detailed autopsies (at a rate of 100 percent in some cases) and new knowledge about the effects of tumors and infectious diseases was readily available. But, fast forward to today and we are largely stuck in a rut with 21st century autopsies performed similarly to the 1900s. We definitely need to move on and that was the thrust of my article published in 1996!

In my view – and I'm going to reiterate my 20-year-old comments because, as I say, so little seems to have changed – autopsies won't become the norm until they can compete with today's requirements for speed and cost-effectiveness. The drive is for efficiency and the cost of performing an autopsy simply to restate the clinician's findings that the patient did have widespread carcinomatosis will no longer suffice in a time where cost benefits are high on the financial agenda.

My proposal still stands that we pathologists need to change our attitude to the autopsy and no longer see it as a thorough study. Instead, we need to take a selective approach. Yes, keep with the Rokitansky tradition when needed, but for the vast majority of cases we can perform "partial" autopsies in a similar way to how surgical pathologists examine and sample surgical specimens. We can, for example, in many circumstances sample sections from the organs of interest, in addition to select metastatic nodules and perhaps a few other organs that appeared abnormal on gross inspection to answer the clinician's questions in short order. And, we should be able to deliver the final autopsy report within 48 hours. Yes, there will be exceptions – but they will be in the minority.

To enable this timelier process we need to approach the autopsy as if it is a surgical specimen. The pathologist will align his or her thinking with the very reason they have the opportunity to do the procedure – because somebody had some questions that need fast, accurate and concise answers. The clinician will be pleased if they get the answers, even partial answers, presented in a two- or three-page report within a few days. They don't need an old-fashioned report numbering 15 pages or

"We pathologists need to change our attitude to the autopsy and no longer see it as a thorough study."

more that go into fine detail about every organ in the patient's body weeks or even months after ordering the autopsy. They simply want the relevant facts and they want them as quickly as possible. Such a partial autopsy will require a great deal of skill and disease knowledge on the part of the pathologist in deciding what to focus on and what to ignore. And, that will no doubt require specialist training and our academic institutions will need to be able to support such teaching.

So, in 1996, I was optimistic that my proposal for a selective, partial autopsy approach should result in an increase in the number of autopsies performed. But, that hasn't been the case. However, I do maintain my optimism as posthumous analysis is the logical way forward; we just need to consider it as the future for our field rather than continue to hold on to our now antique autopsy traditions.

Juan Rosai is the Director of the Milan-based Italian Diagnostic Center's pathology and oncology consultancy. One of the eminent people in the field, he has mentored and taught many surgical pathologists. He is a prolific author with more than 400 scientific papers appearing in peer-reviewed journals. The influential textbook, Rosai and Ackerman's Surgical Pathology, first appeared in 1953 and was published by his mentor, Lauren Ackerman. He continued to publish new editions of the book and the current tenth edition published in 2011. He has characterized novel medical conditions such as Rosai-Dorfman disease and the desmoplastic small round cell tumor.

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The High Price of Diagnostic Error

Putting patient safety first means reducing laboratory-associated and diagnostics errors

By Mario Plebani

I have made it my mission to help increase awareness of "patient safety" in laboratory medicine, and in particular demonstrate the link between laboratory-associated and diagnostic errors. The latter are the leading cause of paid malpractice claims in the USA – twice as many claims than any other type of medical error (1)! Recent data show, among malpractice claims, diagnostic errors are the most common source, most costly and most dangerous of medical mistakes for both inpatients and outpatients (1,2). Diagnostic errors, therefore, are common, produce avoidable disability and death, and yet, worryingly, they remain a relatively understudied and unmeasured area of patient safety (3).

Why this limited recognition? In part it's because they are difficult to define and measure. The best definition for diagnostic errors is, I believe, "errors in which diagnosis was unintentionally delayed (while sufficient information was available earlier), wrong (another diagnosis made before the correct one), or missed (no diagnosis made) as judged from the eventual appreciation of more definitive information (for example, autopsy studies)" (4). The etiologies of such errors are numerous and the sources categorized as "cognitive", "system-related" or "no-fault" factors (4) – and laboratory errors play a fundamental role in this context.

The evidence for the importance and direct link between diagnostic errors and errors in laboratory medicine derives from a series of studies that have a clinical starting point. In particular, studies performed on the pre-preanalytical phase confirm that failure to request appropriate diagnostic tests (laboratory tests included) makes up 55 percent of missed and delayed diagnoses in the ambulatory setting (5) and 58 percent of errors in emergency departments (6). In the final stages of the test loop, incorrect interpretation of diagnostic or laboratory tests cause a large percentage of errors in the ambulatory setting and in emergency departments (7). For example, failure to inform patients of clinically significant abnormal test results or to record the delivery of relevant information is relatively common, and has been found to occur in one out of every 14 tests. The overall rate of failure to inform the patient or to record communication of information is 7.1 percent, and in different practices, it ranges from zero to 26 percent (8).

And the evidence continues to mount… The literature shows failure to follow-up test results markedly compromises patient safety, yet the rate of abnormal laboratory results without follow-up (for INR [international normalized ration] and PSA [prostate specific antigen]) ranges from 6.8 to 62 percent! (7). Further evidence of erroneous reactions to laboratory information is provided in a study evaluating the prescription of potassium in cases of hyperkalemia (9). And, findings in another study (10) showed that over 2 percent (2.6 percent in 2000, 2.1 percent in 2007) of patients with thyrotropin (TSH) levels exceeding 20 mU/mL were not followed up. Yet another study revealed that of 1,095 discharged patients, almost half had pending laboratory and radiology test results, 9 percent of which were potentially actionable (11).

Overall, data reported demonstrate that the initial and final steps of the total testing process, above all test requesting and reaction to laboratory results, are not only more error-prone than all the other steps, but are also the most important causes of potential adverse outcomes for patients. The data also confirm that a relevant number of failures occur in the interface between clinicians' clinics and laboratories, which emphasizes the need for closer cooperation and interaction at the clinical-laboratory interface (12).

The current nature of laboratory testing-associated errors – in particular the link between appropriateness in test ordering and result interpretation/utilization – and their potential in addressing diagnostic errors, should herald a change in the old paradigm which focused only on errors detected within the laboratory walls (13–15). Translating the concept of "patient-centered care" from theory to practice is essential for investigating, and improving, not only all procedures and processes performed under the direct control of the clinical laboratory, but also the initial and final steps of the testing cycle that are usually managed by other healthcare operators. In my experience, a fundamental tool for allowing the identification and minimization of errors in the total testing process is the development of a harmonized model of quality indicators, such as that proposed by a Working Group of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (15,16).

Mario Plebani is a full professor of Clinical Biochemistry and Clinical Molecular Biology at the School of Medicine, University of Padova, Italy. He is also chief of the Department of Laboratory Medicine at the University-Hospital of Padova, chief of the Center of Biomedical Research (Veneto Region). As well as his academic, medical and management duties in Padova, he is an honorary professor at the University of Buenos Aires (Argentina). His main areas of research are quality in laboratory medicine, diagnostic and laboratory errors, biomarkers in cancer and cardiovascular diseases, and in vitro allergy diagnostics.

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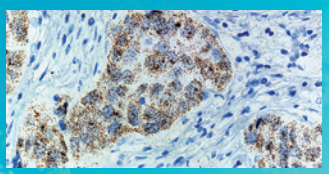

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Navigating 21st Century Virology

Virology remains a rapidly changing field and the diagnostic clinical laboratory must keep abreast of newer viruses, targets and technologies to provide accurate, cost-effective and timely results for patients. Emerging diagnostics promise to change the field forever – are you ready?

By Malur Sudhanva

Back in the 1950s and 60s, routine virology diagnostics in teaching hospitals involved virus isolation in cell lines and serology, using techniques such as complement fixation tests. By the 1970s and 80s, electron microscopy and immunofluorescence techniques were firmly planted at the forefront of testing. Then the huge discovery of HIV in the 1980s paved the way for the development of enzyme immunoassays. This was followed in the early 1990s by the introduction of the popular polymerase chain reaction (PCR) assays. Nowadays, the availability of several new molecular techniques, most of them automated, is a legacy of three decades of nucleic acid research. But given the vast range of options currently available, it's unsurprisingly difficult for today's clinical virologist to select the techniques and technologies to best suit their needs.

Bring on the next generation

Let us begin with a technique that broke new ground and opened up a whole new world of scientific discovery and knowledge: capillary electrophoresis-based Sanger sequencing. This practice-changing method has enabled scientists to elucidate genetic information from any given biological system for almost 40 years now, and it's still routinely used by virology laboratories for HIV and hepatitis B virus (HBV) sequencing. However, Sanger sequencing has fairly substantial limitations in the human genomics field in terms of throughput, scalability, speed and resolution. In a bid to address these limitations, next-generation sequencing (NGS) technology came to the fore. NGS is now a catch-all term to describe a number of sequencing technologies, many of which hold potential in routine diagnosis (see Figure 1).

NGS applications within virology have been summarized as (1):

i) detection of unknown viral pathogens and discovery of novel viruses,
ii) detection of tumor viruses,
iii) characterization of the human virome,
iv) sequencing of full-length viral genome,
v) characterization of viral genome variability and viral quasispecies,
vi) monitoring antiviral drug resistance, epidemiology of viral infections and viral evolution and quality control of live-attenuated viral vaccines.

But with a diverse range of technologies, the question remains: how useful are they in routine diagnostic virology? Published results for each system show them to be largely interchangeable (1).

A collaborative effort across Public Health England (PHE) sites is in the process of validating NGS for HIV-1 genotypic resistance testing with a view to fully replacing Sanger sequencing with MiSeq (Illumina); however, this has not yet been published. As with other NGS studies, it is expected that the assay threshold could be drilled down to one percent of minority species compared with 10–15 percent using Sanger sequencing. But the clinical benefit of detecting minority variants by NGS need to be evaluated both in the short and long term (2).

Fortunately, the impact of NGS in diagnostic virology involving HIV-1 tropism, genotypic resistance in influenza, HBV, hepatitis C virus (HCV) and cytomegalovirus (CMV) has already been summarized (3). Though the authors noted benefits including "increased sensitivity and eventually cheaper antiviral resistance tests," they cautioned, "there is a risk that low percentage minority variants may be over interpreted. This could result in antiviral drugs, which may have been effective, being possibly denied to patients if proper clinical validation studies are not performed." Furthermore, numerous papers from around the world have reported on the use of NGS technology in virus outbreak monitoring and mapping quasispecies variations; for example, influenza virus, hepatitis A virus, norovirus, enterovirus, and ebola virus during the 2014 West African outbreak (4, 5, 6, 7, 8).

Despite numerous publications demonstrating the advantages of using NGS in virology, its uptake has been severely slowed by the lack of widespread collaborations between human/cancer geneticists and virologists; the cost of equipment; and the continued availability of the well-established Sanger sequencing method for routine nucleic acid sequencing. As a result, NGS has not really taken off in the diagnostic clinical virology field, yet (2, 3). But that could all change within months – once the cost- and clinical-effectiveness of the relevant technologies are established.

Automation of liquid handling

In the new millennium, reagent volumes have decreased, but testing workload has increased. It is now well recognized that manual pipetting is a liability that increases the likelihood of original sample contamination and mix-ups. Whether it is enzyme immunoassay or nucleic acid extraction, repetitive steps demand precision. So it's understandable that the preferred option these days is to decrease manual repetitive tasks and automate, wherever possible.

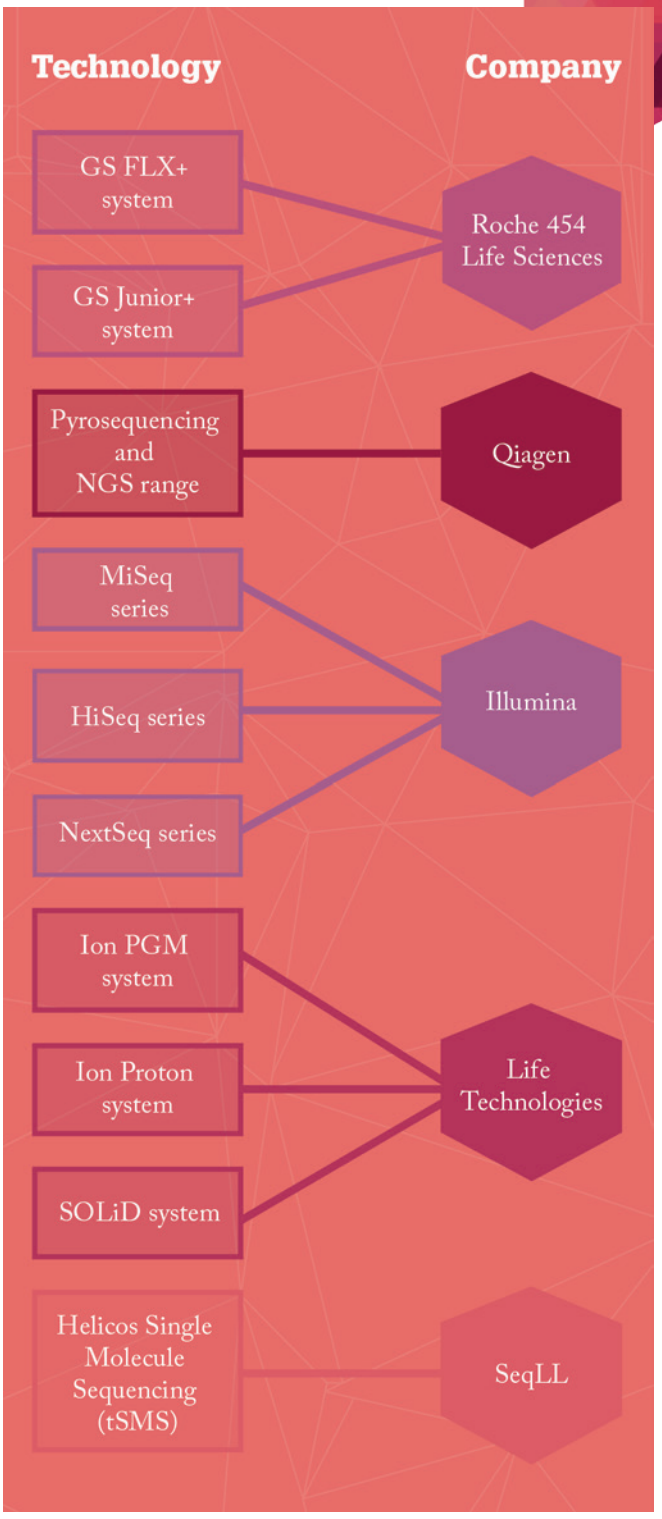

Figure 1. Some of the NGS systems that hold potential in routine laboratory diagnosis.

Evolution of virology diagnostics

1892 Dmitry Ivanovsky becomes the first man to discover a virus after experimenting with filters with pores too small to retain bacteria (1).

1909 Introduction of the complementation fixation test for treponema causing syphilis. Despite success, the test took a number of decades before it was adapted for general virology / bacteriology (2).

1925-1950 Animals used for virus isolation and detection including monkeys, mice and embryonated eggs (3).

1940 The first visualization of a virus using an electron microscope, subsequently leading to the discovery of many viruses (4).

1949 Cell lines introduced for virus diagnostics for the first time, successfully isolating polio virus and providing a more humane method than animal isolation (3).

1956 Immunofluorescence used for the first time to identify influenza viral antigen, soon becoming used as a diagnostic in many laboratories (5).

1971 Enzyme-linked immunosorbent assay (ELISA) is developed, allowing highly sensitive detection of antibodies and antigens (6).

1983 Scientists discovered HIV as the causative agent of AIDS, accelerating virus diagnostic development (7).

1983 PCR was revealed to the general public. Over the next few years, patents would be obtained and the amplification process would solidify itself as common place within the laboratory (8).

1986 Taq polymerase was isolated and became an important part of PCR, however, it's lacking proofreading capabilities proved problematic (9).

1992 Strand displacement amplification (SDA) developed allowing an alternative to PCR, whilst working at a constant temperature (10).

1994 Additional DNA polymerases, with proofreading abilities, were introduced into a PCR blend with Taq, such as Pwo polymerase (9).

2000 Loop-mediated isothermal amplification (LAMP) is developed and is capable of amplifying DNA at a considerably higher rate than conventional PCR (11).

2003 The introduction of fusion DNA polymerase which could overcome many factors limiting PCR, including stability and strong proofreading capabilities (12).

2006 First commercial system for digital PCR introduced (papers on the method had been published as far back as 1992) (13).

2008 Electrospray ionization mass spectrometry (ESI-TOF) is utilized for broad-spectrum pathogen diagnostics (14).

2010 Microbial Detection Array developed to meet the growing demand for broad-spectrum pathogen detection (15).

2014 Lawrence Livermore Microbial Detection Array (LLMDA) is thought to improve on previous methods introduced in 2010, providing a wider range of detectable pathogens (16).

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Liquid handling for nucleic acid extraction has come a long way since the 1990s when manual mixing of PCR reagents was the norm. The diagnostic market is now flooded with liquid-handling machines, each generation being an improvement over the last, both in capacity and speed. They are also constantly evolving to accept different types of samples, process lower volumes suitable for generic and/or manufacturer's own specific reagents, and dispense liquids into 96-well plate format/circular disc/other topographical variations. Most machines have a bi-directional interface with a laboratory information management system (LIMS) and some have evolved to become a complete solution macro-robot or miniaturized technology using microfluidics (see below). Roche, Qiagen, Tecan, Abbott, Siemens, Beckton Dickenson and Life Technologies are just some of the major manufacturers in this field.

Automated liquid handling technologies are clearly beneficial, but there are instances when manual methods are still preferred, specifically when dealing with precious low volume samples, like pediatric blood, vitreous fluid and CSF samples, which still need initial manual processing to prevent 'dead volume' loss in automation. As a general rule, when sample numbers are low in a laboratory, capital investment in automation equipment is not cost-effective; but as sample numbers increase, so does the return on investment.

"This has substantially improved capacity within the lab, as well as efficiency and the reproducibility of assays."

The Rare and Imported Pathogens Laboratory (RIPL) in Porton Down, Microbiology Services Public Health England (PHE), UK, for example, has replaced all manual pipetting processes in indirect immunofluorescence assays with the IF Sprinter (Euroimmun), thus fully automating the process – from dilution and dispensing of samples to incubation and washing of microscope slides. The upgrade has resulted in increased capacity and decreased manual pipetting errors and inter-operator variations.

Further, the entire molecular virology setup, from nucleic acid extraction to detection, at South London Specialist Virology Centre, King's College Hospital in London, has been automated since 2008. A constant challenge in virology

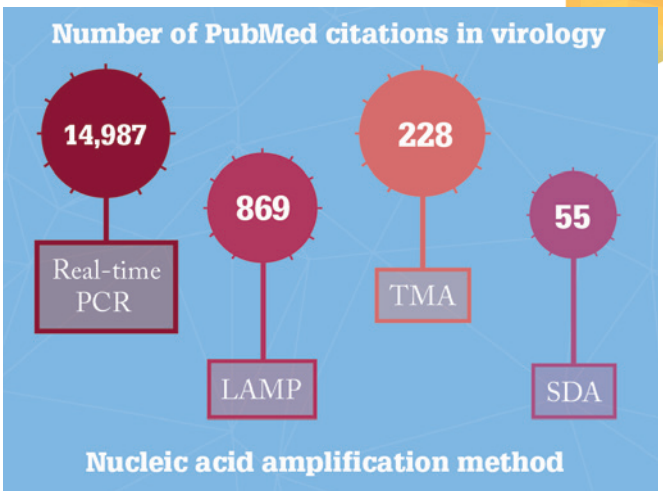

Figure 2. Comparing select nucleic acid amplification methods by number of research citations in virology. PCR, polymerase chain reaction; LAMP, loop mediated isothermal amplification; TMA, transcription mediated amplification; SDA, strand displacement amplification.

is that one can never predict a sudden increase in workload, such as the 2009 pandemic influenza virus. During the initial few weeks of this pandemic, we were inundated with 800 respiratory samples per day for influenza A virus RNA testing, which stretched our automation to its limit.

In 2013, we installed the Freedom EVO platform (TECAN) to separate serum and plasma for the extraction of RNA and DNA and for liquid dispensing into various sample racks, plates and capillaries. Earlier variations of this platform are still in use and they perform the following tasks: sample aliquoting, sample dilution, complement fixation test (CFT) plates, agglutination assays, ELISAs and final aliquoting for sample storage. All samples are tracked via barcode, and all worksheets are linked to the LIMS. This has substantially improved capacity within the lab, as well as efficiency and the reproducibility of assays.

The latest in PCR and its rivals

Nucleic acid amplification tests have been the focus of much development since the mid-1990s because of the sensitivity, specificity, turnaround time (TAT) and contamination issues surrounding the now redundant block-based assays. Today in the commercial world, thermal processes like PCR compete with isothermal processes like transcription mediated amplification (TMA), loop mediated isothermal amplification (LAMP), strand displacement amplification (SDA), and so on, in terms of lab spend. Nevertheless, real-time PCR forms the backbone of molecular diagnostic virology processes worldwide. A simple PubMed search using the term "real time

Table 1. The ideal Point-of-Care Test according to WHO ASSURED criteria (12).

A	Affordable
S	Sensitive
S	Specific
U	User-friendly (simple to perform in a few steps with minimal training)
R	Robust and rapid (results in less than 30 minutes)
E	Equipment-free
D	Deliverable to those who need them

PCR virus" led to a listing of nearly 15,000 articles with the first article dated 1993 (9), and this is in the narrow field of virology (Figure 2). Since then, new developments in real-time PCR have yielded improved sensitivity, probe-based specificity, increased capacity to multiplex and differentiate (because of the availability of numerous fluorescent dyes – from six to 30 over the last decade), and increasing availability of analytical software. Today, assays can be developed rapidly and reagents can be purchased for as little as £1 per reaction.

Though impressive and extremely useful in virology testing, nucleic acid amplification sequences one or a small set of organisms to rapidly identify selected pathogens at the species or strain level, but cannot be multiplexed to the degree required to detect hundreds to thousands of different organisms. A downfall that has been addressed by the new Lawrence Livermore Microbial Detection Array (LLMDA) (10). LLMDA is a multiple displacement amplification (MDA)-based whole genome amplification, which uses Phi29 polymerase, known to offer high processivity and low error rate when compared with Taq polymerase. It can be used for whole transcriptome amplification with 2.1 million probes available representing different pathogens. The authors of the original research have now successfully used LLMDA to detect a range of emerging viruses, including dengue virus, West Nile virus, Japanese encephalitis virus, tick-borne encephalitis virus, yellow fever virus, to name but a few.

A wide variety of automated liquid handling platforms are available for running real-time PCR and other nucleic acid detection techniques, many of which can accommodate both commercial and in-house tests. Virologists have accepted and adapted real-time PCR, resulting in decades of accumulated collective experience and confidence in its results. It was only a matter of time before miniaturization and microfluidics took over, paving the way for the first ever fully integrated and automated nucleic acid sample preparation, amplification, and real-time detection system (11). Released by diagnostics firm Cepheid in 2007, the system consists of an instrument, a personal computer, and disposable fluidic cartridges. The ease of use of this instrument has led to its application as a molecular point-of-care test (POCT).

POCT on the up

There is now widespread use of POCTs in clinics and in the field – for example, antigen detection (influenza and respiratory syncytial viruses), antibody detection (HIV and HCV) and cell counts (hemoglobin, neutrophils and CD4 count). A great deal of research and development has focused on POCT in recent years and this trend is showing no signs of relenting. If anything, it is accelerating. Today, a number of commercial companies now operate in the molecular POCT sector, developing technologies that range from cartridge-, membrane-, and microarray-based solutions to isothermal and thermal molecular systems. Some are just entering into human clinical trials and others have tremendous potential. In 2001, the World Health Organization outlined the key – or so-called "ASSURED" – criteria that each POCT must meet to be deemed viable (see Table 1) (12).

Some of the available technologies have been summarized in Table 2, with a new proposed rating based on this very practical ASSURED criteria. In this proposed rating, presence of each attribute within the ASSURED criteria gets a score of 1, with a maximum possible score of 7. For the sake of simplicity, in this article, it is assumed that all these molecular technologies are sensitive and specific enough to warrant a starting baseline score of 2 out of a maximum possible 7 in the ASSURED criteria.

The companies that now operate in this arena vary widely in their expertise and technological approach. Theranos, for example, has been a pretty secretive company, having not released a single photograph of its equipment or published any data in peer-reviewed journals (13, 14). The two papers published (as highlighted in Table 2) did not originate from the manufacturing company. However, the US-based tech firm has recently received a welcome boost by US FDA regulators who have been wooed by Theranos' technology; the firm has been cleared to market its herpes simplex 1 virus IgG (HSV-1) nationally, and it's also received a highly sought after Clinical Laboratory Improvement Amendments (CLIA) Waiver, permitting use of it in locations outside of traditional clinical laboratories. How did they manage it? According to their press statement, "Theranos provided study data from 818

Technology	Affordable*	User-friendly**	Rapid	Free of additional equipment	Deliverable***	Proposed ASSURED score (maximum = 7)	Comments	Number of PubMed citations
i System (Alere)	Yes	Yes	Yes	No	Yes	6	Influenza, malaria and HIV assays available. Fully adaptable to resource-poor settings and test result returned in less than 15 minutes.	35
io System (Atlas Genetics)	Yes, but unable to confirm	Yes	Yes	No	Yes	6	Specifically designed for use in decentralized laboratories and point-of-care settings to detect infectious disease, such as chlamydia and MRSA in around 30 minutes.	0
BD Max System (BD Diagnostics)	No	Yes	Yes	No	Yes	5	Bacterial and viral assays including C. difficile, MRSA and Group B Streptococcus. More assays coming soon. Can run multiple specimen types and assays in a single run; designed for laboratories.	29
Biomeme (Biomeme)	Yes, but unable to confirm	Yes	Yes	Yes	Yes	7	Combines iPhone 5s technology with sample prep system to perform real-time PCR. Returns results in less than 1 hour at point of care. A panel of tests currently exist for sexually transmitted infections.	0
FilmArray (Biomerieux, BioFire)	No	Yes	Yes	No	Yes	5	Relatively expensive to own and run, but small footprint, respiratory, bloodstream and gastrointestinal panels available. Returns results in around 1 hour; each panel testing for 20+ pathogens. More assays coming soon.	27
GeneXpert (Cepheid)	Yes	Yes	Yes	No	Yes	6	First in the market. Extensive set of panels. Fully integrated and automated systems with multiple configurations. Proven in resource-poor settings.	50
ELITe InGenius (ELITechGroup)	No	Yes	Yes	No	Yes	5	Designed for laboratories, it integrates sample preparation, amplification and result analysis, validated with a transplant pathogen monitoring menu (currently in development). No virological assays available yet.	0
Enigma ML (Enigma Diagnostics)	Yes, but unable to confirm	Yes	Yes	No	Yes	6	System developed for rapid point of care testing with simple and multiplexed assay formats. Influenza and respiratory virus assay available with others in development.	1
Extreme PCR (The Wittwer DNA Lab, Utah University)	No	Yes	Yes	No	Yes	5	Prototypes only. Huge potential, no assays.	1
Xtreme Chain Reaction (XCR) (Fluoresentric)	Yes, but unable to confirm	Yes	Yes	Yes	Yes	7	Multiple virus assays, including Zaire ebola virus,, influenza, norovirus, rotavirus, MERS, HBV assays available. Designed for point-of-care testing with results returned in 5 minutes.	1
Simplexa (Focus Diagnostics)	Yes, but unable to confirm	No	Yes	No	Yes	5	Designed for laboratories, requires centrifuge and a liquid handler. Numerous virus assays available, including CMV, adenovirus, herpes, and Epstein-Barr.	23
eSensor XT-8 (Genmark Dx)	No	Yes	Yes	No	Yes	5	Similar to BioFire, but more flexible, in my opinion. Designed for laboratory use. Respiratory viral panel only available currently. More assays coming soon.	6
Portrait (Great Basin Scientific)	Yes, but unable to confirm	Yes	Yes	No	Yes	6	Bacterial panels only currently available, with others in development. 90 minute wait for results.	0

Table 2. A summary of the range of potential and established molecular POCTs in virology. A new proposed POCT rating considering the presence of each attribute within the ASSURED criteria (12) (see Table 1) is included. Each attribute presence gets a score of 1, with a maximum possible score of 7. It is assumed that all of these molecular technologies are sensitive and specific enough to warrant a baseline score of 2.

Technology	Affordable*	User-friendly**	Rapid	Free of additional equipment	Deliverable***	Proposed ASSURED score (maximum = 7)	Comments	Number of PubMed citations
ARIES (Luminex)	No	No	Yes	No	No	3	Designed for laboratories, but the platform appears to be adaptable, easily scalable and robust. Designed to run multiple assays simultaneously, the system will be available soon.	0
illumigene (Meridian Bioscience)	Yes, but unable to confirm	Yes	Yes	No	Yes	6	Employs Loop-Mediated Isothermal Amplification (LAMP) technology for use in laboratories, providing up to 10 sample results in less than 1 hour. Bacterial assays only currently available.	18
Magnetic Integrated Microfluidic Electrochemical Detector (MIMED) (University of California)	Yes, but unable to confirm	Yes	Yes	Yes	Yes	7	A point-of-care test that integrates sample preparation and electrochemical sensors in a disposable device to detect RNA-based viruses. It can currently detect influenza H1N1 in throat swab samples at low loads.	1
PanNAT (Micronics)	Yes, but unable to confirm	Yes	Yes	Yes	Yes	7	A point-of-care system that delivers results in less than 1 hour, without the need for sample prep. Designed for decentralized environments. No assays, yet.	4
Verigene Processor SP (Nanosphere)	No	Yes	No	No	Yes	4	Employs gold nanoparticle probe technology. Designed for laboratories, bacterial and viral infection assays available targeting blood-borne, respiratory and gastrointestinal infections. Two or more hours to results; good panel, in my opinion.	48
Palm PCR (Ahram Biosystems)	Yes, but unable to confirm	Yes	Yes	Yes	Yes	7	Battery-powered, palm-size, portable PCR machine. Amplification within 30 minutes, designed to conform to the standard 9 mm-spacing well format to use with a disposable plastic sample tube. No assays, yet.	1
Q-POC (QuantuMDx)	Yes, but unable to confirm	Yes	Yes	Yes	Yes	7	Returning results in 15 minutes with a fingerprick of blood, this handheld, smartphone-like device is currently undergoing testing to diagnose infectious diseases, cancer and pharmacogenetics. No assays, yet.	1
Amplivue (Quidel)	Yes, but unable to confirm	Yes	Yes	Yes	Yes	7	Using a small hand-held cartridge that requires no equipment beyond a thermal block with a heated lid, this is designed to be cost-effective and efficient. Mostly bacterial assays are currently available, only viral assay is for HSV DNA currently.	5
EncompassMDx (Rheonix)	Yes, but unable to confirm	Yes	Yes	Yes	Yes	7	Operates interchangeable purification, amplification and detection modules organized on single-use cartridges. Product development programs for infectious diseases, pharmacogenomics and environmental applications are ongoing.	5
Cobas Liat - Lab in a tube (Roche)	Yes, but unable to confirm	Yes	Yes	Yes	Yes	7	Designed for laboratory and point-of-care testing, the system includes a benchtop analyzer and assays for infectious diseases, including Influenza A/B and respiratory infections.	3
T-COR 8 (Tetracore)	Yes	Yes	Yes	No	Yes	6	Used in biothreat and veterinary field, very adaptable, cloud-based and portable, weighing less than 4.5 kg.	11
Theranos (Theranos)	Yes	Not sure	Yes	Not sure	Yes	5	Using finger sticks collected from subjects, this test has been approved in the US in labs and outside of certified laboratories. Multiple viral assays available, including respiratory influenza virus assay and herpes simplex virus assay.	2
Firefly Dx (PositiveID)	Yes, but unable to confirm	Yes	No	No	Yes	5	Handheld system with a series of cartridges for biological sample processing and detection, providing results in less than 20 minutes at the point of need.	1

* Based on a proposed new scoring ** Based on manufacturer's literature *** Tests deliverable to end-users

subjects of varying age and ethnicity, demonstrating that its system could be run accurately using only a finger stick as well as a traditional venous draw across large numbers of Theranos devices, all compared against an FDA cleared, commercially available reference method". Other manufacturers, like Tetracore (15), have ventured only recently into human pathogen detection, and like Theranos, there are some firms that have also produced an array of user-friendly products for the laboratory, which can also be used in the POCT setting (BioFire FilmArray and Genmark Dx eSensor, for example) (16, 17).

With regards to technological capabilities of these tests, some incorporate new PCR methods, like Extreme PCR, developed by The Wittwer DNA Lab in Utah University, which has a reaction time between 15 and 60 seconds (18). Others have adapted existing reagent technologies like Xtreme Chain Reaction and Biomeme, but using smartphone capabilities (19, 20). The number of PubMed citations listed in Table 2 is an indication of the maturity of the technology and its acceptance within the medical field. Though the list of POCTs in the table is by no means exhaustive, it is indicative of the technological developments taking place in this field; any one of these technology types has the potential to be a diagnostic game-changer.

It's all going digital

As with many fields of pathology, digitization is now revolutionizing the field of PCR (21). This latest refinement of conventional PCR can be used to directly quantify and clonally amplify nucleic acids. Like PCR, digital PCR carries out one reaction per single sample; however, the sample is separated into a large number of partitions – either as a series of droplets or split into nanoscale reaction wells – and the reaction is carried out in each partition individually. The outcome? High sensitivity and precision, high tolerance to inhibitors and amenability to quantitation. This allows a more reliable quantitation of nucleic acids like CMV quantitation standards (22). Research has been published on its use in Chlamydia trachomatis detection in trachoma cases, as well as identification of rotavirus in water, HIV-1 proviral DNA and HHV-6 DNA (23–26). However, the upfront and assay validation costs associated with digital PCR make its potential role in routine diagnostics unclear, especially given that the real-time PCRs are already well established in the diagnostic laboratory.

Mass spec prospects

Taking a different approach, Abbott is combining sample preparation, broad PCR amplification, and electrospray ionization mass spectrometry (ESI-MS) of DNA amplicons in an automated platform to identify base composition based on molecular weight (27, 28). Previously, this technology was known as Ibis T5000 and Abbott PLEX-ID, but it was re-launched in 2015 as IRIDICA. With this new technology, bacteria, viruses, fungi, and protozoa can be screened against a library of more than 750,000 entries to perform high-resolution subtyping, identification of known virulence markers and antibiotic resistance genes, and identification of mixtures of microbes from a single sample. Currently, Abbott has focused the development and marketing of IRIDICA within bacterial sepsis, mycology and sterile fluid diagnostic bacteriology. A panel for plasma viruses with further panels for respiratory viruses and encephalitis viruses exist and these could potentially become competitive against established real-time PCR if the pricing is pitched right.

"Personally, I believe the future lies in further automation and miniaturization of enzyme immunoassays and nucleic acid amplification, providing either qualitative or quantitative results."

Looking to the future

There are a huge number of technologies that could provide real value to the clinical virology laboratory, especially when they are fully automated. Though I haven't covered them all in this article, I believe it's clear that there are some exciting developments ahead that will revolutionize the way we diagnose viral diseases. Personally, I believe the future lies in further automation and miniaturization of enzyme immunoassays and nucleic acid amplification, providing either qualitative or quantitative results.

Perhaps the hospital laboratory will evolve to support a syndrome-based POCT service by developing community-based near-patient-testing projects so that family physicians and community nurses have access to the technology. For decades, physicians have sent samples away to the laboratory for testing and awaited results and interpretation. Future physicians are likely to interpret patient test results on their own and follow set clinical pathway protocols, thereby

hopefully decreasing the overall cost of hospital stay. In fact, this progression is already happening in places where molecular POCTs have been installed in hospital wards. Once these technologies are widespread and interconnected, a truly integrated healthcare system is possible.

Where would hospital-based laboratory testing fit? It makes sense that laboratory medicine expertise and technologies be directed towards the management of more complex and multiple pathogen infections, novel pathogen discovery in idiopathic syndromes, providing high-volume tests at low cost, delivering automated algorithm-based interpretation of difficult sets of results and widespread real-time use of genome sequencing for infection control purposes. There is no reason why POCT and laboratory diagnostics could not co-exist in a mutually beneficial way. It's already happening in some hospitals and my prediction is that it will only continue to become increasingly standard in the months and years ahead.

Malur Sudhanva is consultant virologist at South London Specialist Virology Centre, Viapath, King's College Hospital NHS Foundation Trust, London, UK and honorary consultant virologist at Rare and Imported Pathogens Laboratory, Public Health England Microbiological Services, Porton Down, UK.

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Unlocking the Value of RNA

RNA biomarkers lag behind DNA and proteins in clinical use because of the limitations of lab technology – but new analytical tools could now make RNA a useful tool in the pathologist's kit

By Xiao-Jun Ma

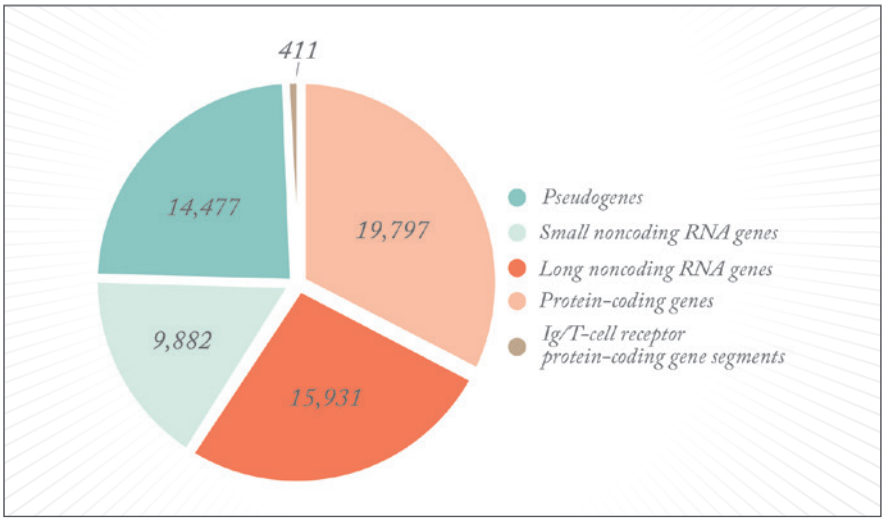

Figure 1. Current understanding of the division of human genes between protein-coding, RNA, immunoglobulin/T-cell receptor gene segments and pseudogenes.

Our understanding of RNA is constantly evolving, with exciting discoveries like alternative splicing and RNA catalytic activity only a few decades old (1). The discovery of noncoding RNAs has opened up a new world of nucleic acid function, helping us to understand how life's incredible phenotypic diversity arises from a relatively small, fixed set of genes. Amazingly intricate and dynamic, alternative splicing has the potential to produce any number of splice variants for a given gene. In fact, according to the most recently published human genome (2), we now have approximately 60,000 human genes transcribing into nearly 200,000 RNA species (see Figure 1 for a more detailed breakdown). The expression levels of those RNAs are highly dynamic, integrating both genetic and epigenetic mechanisms of gene regulation to reflect the state of a biological system – which makes them an excellent choice as biomarkers.

Biomarkers, indicators of biological and disease states, have long been used for diagnostic testing – and in this era of personalized medicine, their importance is growing exponentially. All three of the main macromolecules in the cell – DNA, RNA and protein – can serve as valuable markers of a specific biological trait or measurable change directly associated with a physiological condition or disease status (3). But measuring the biomarkers themselves can prove challenging, as not all of the tools for doing so are created equal in terms of information obtained, technical advances or clinical compatibility.

Which biomarker is best?

Most biomarkers today are discovered as RNA, thanks to transcriptomic discovery programs that rely on the high-throughput capabilities of microarrays and RNA sequencing. Because RNA profiling benefits from rapid and relatively inexpensive next-generation sequencing (NGS) and whole-genome microarrays, RNA is an ideal biomarker choice – and yet, it remains difficult to translate discoveries to the clinic for routine measurement. Why? The blame lies with established analytical technologies. Although it's commonplace to detect and visualize native DNA and proteins in a single cell, the best routine measurement tools for RNA analysis detect and quantify it in solution, which results in a loss of morphological context. Because we lack appropriate methodology for measuring RNA *in situ*, many laboratories use DNA and protein as surrogates, which can be problematic for a variety of reasons (see Table 1).

The main issue with analyzing a protein in place of an RNA biomarker is that, because of gene regulation at the transcriptional and post-transcriptional levels, protein and RNA levels rarely exhibit a linear correlation. So even though the expression pattern of an RNA molecule may indicate a particular biological state, its protein counterpart

At a Glance

- *Thanks to transcriptomic discovery programs, researchers have discovered that RNA is a rich source of biomarkers*
- *Because of issues with DNA and protein surrogates, a direct path from RNA biomarker discovery to the clinic is highly desirable*
- *Though it's ideal to measure RNA biomarkers in situ, the tools for doing so have traditionally lacked sensitivity, specificity and ease of use*
- *New multiplex in situ hybridization addresses these obstacles, providing cellular context for accurate analysis of RNA biomarkers*

Biomarker	Pros	Cons
RNA	• Integrates genetic and epigenetic mechanisms of gene regulation • In situ measurement techniques are becoming more accessible • Many biomarkers are discovered as RNA from transcriptomic studies • Expression reflects the state of a biological system • Can detect biomarkers for which no antibodies are available	• Compared to DNA and protein, RNA is more susceptible to degradation during specimen handling and storage, demanding more robust method of detection • RNA work requires special care in the laboratory to ensure an environment free of ubiquitous ribonucleases
DNA	• Single-copy in situ detection is possible through FISH • Can detect structural alterations (translocations, deletions, amplifications) • Specificity is easy to verify visually • NGS offers many benefits	• DNA alterations don't always lead to RNA or protein expression changes • Conventional FISH can't detect DNA alterations at single-gene resolution and is unsuitable for detecting small DNA alterations (microdeletions, micro-amplifications, gene rearrangements) • NGS requires sophisticated bioinformatics and offers no morphological context
Protein	• Functional components of a cell • Well-established methodologies are available, rapid and easy to perform • In situ detection for routine use is available • Options for automation exist • Can be cost-effective when antibodies are available	• Limited antibody availability and quality • Development and validation of new antibodies can be slow and expensive • Limited detection of secreted and scarce proteins • Only works on protein-coding genes • Unsuitable as a surrogate for RNA

Table 1. The pros and cons of using RNA, DNA and proteins as biomarkers.

may signal something very different. And for noncoding RNA molecules, which have no protein counterparts, measuring RNA is the only option. An additional complication is that most protein detection techniques – such as immunohistochemistry (IHC), the main method of protein biomarker detection *in situ* – rely on the use of antibodies, whose quality can be limiting or prevent the detection of scarce or secreted proteins. In some cases, no

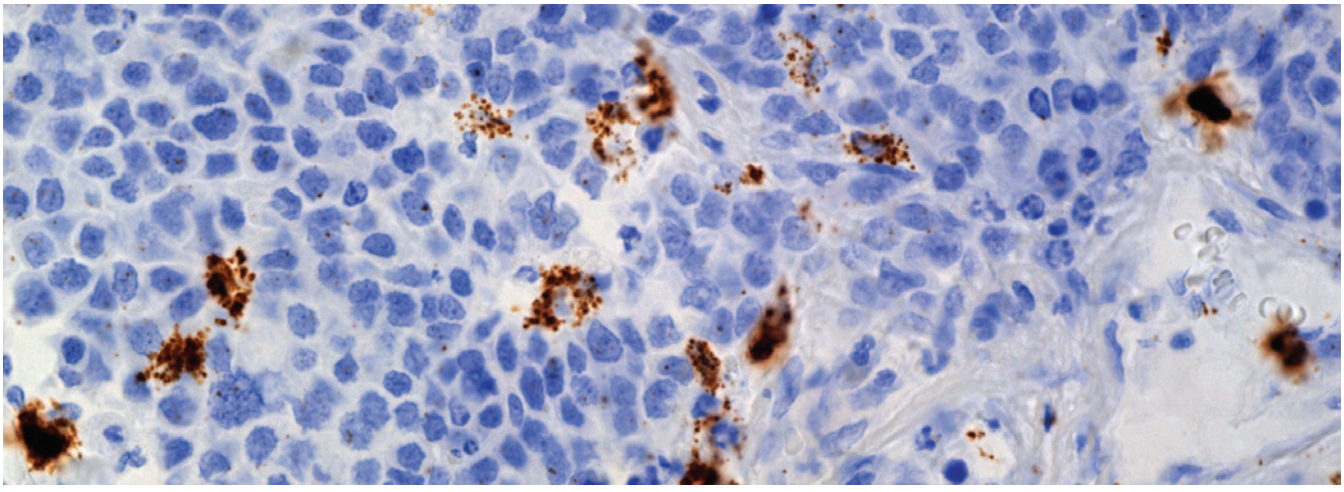

Figure 2. Human breast cancer tissue probed for expression of the MMP9 mRNA, which codes for a matrix metalloprotease whose overexpression facilitates tumor growth and invasion. The signal (brown) is abundant in scattered stromal cells of the tumor microenvironment, but is rarely expressed within the tumor cells themselves (12).

antibody to the protein of interest exists at all, meaning that researchers must either develop and validate their own over time, or find an alternative detection method.

Like RNA and proteins, DNA biomarkers are also important in research and clinical applications. This is especially true because many structural alterations to DNA are linked to cancer and other diseases, so it's important to look at the DNA itself using techniques like DNA fluorescence *in situ* hybridization (FISH), polymerase chain reaction (PCR) and evolving NGS technologies. But structural rearrangements don't always result in changes to transcriptional activity or levels of expression – so an increased DNA copy number does not necessarily translate to an increased amount of RNA or protein (4).

As DNA and protein surrogates may not recapitulate the information provided by an RNA biomarker, and solution-based RNA analysis takes the expression out of its cellular context, what's the best way for laboratory medicine professionals to perform routine biomarker profiling following transcriptomic exploration? The answer lies in direct RNA measurement *in situ*. And at the moment, there's an unprecedented demand for effective tools to do just that. To gain biological insights, we need the ability to not only detect the RNA, but also to localize it in the cell. It's this combination of detection and localization that researchers are targeting now in order to take advantage of RNA's potential as an indicator of health and disease.

The key to precise localization

First applied to RNA in 1981 (5,6), *in situ* hybridization (ISH) can effectively determine the precise localization of target RNA in single cells. The technique provides the spatial and morphological context we need to understand the physiological and pathological relevance of a given target RNA. For instance, we can detect RNA expression in specific cell types to distinguish between stromal and tumor expression (see Figure 2), or detect it in rare cell populations such as cancer stem cells or circulating tumor cells. So if the answer to the RNA measurement conundrum lies in a technique that was introduced decades ago, why is it not widely used? In the past, ISH was not only time-consuming and difficult to use, but it lacked sensitivity and specificity – deficits that presented a barrier to its becoming commonplace in research and diagnostic pathology labs. But now that there have been some significant advancements to ISH techniques, the outlook is changing.

Nowadays, we have multiplex nucleic acid *in situ* hybridization technologies that overcome those limitations, enabling single-cell gene expression profiling *in situ* with detection sensitivity and specificity down to a single molecule (see Figure 3). Incorporating a variety of innovative approaches to probe design and signal amplification, these recent advances (7–11) take *in situ* RNA detection to an entirely new level. Single-molecule detection allows single-cell transcript quantification to be as simple as counting dots in a cell, which can be automated with advanced image analysis software. Robust probe design strategies also provide compatibility with partially degraded RNA, allowing for robust RNA detection in routine clinical

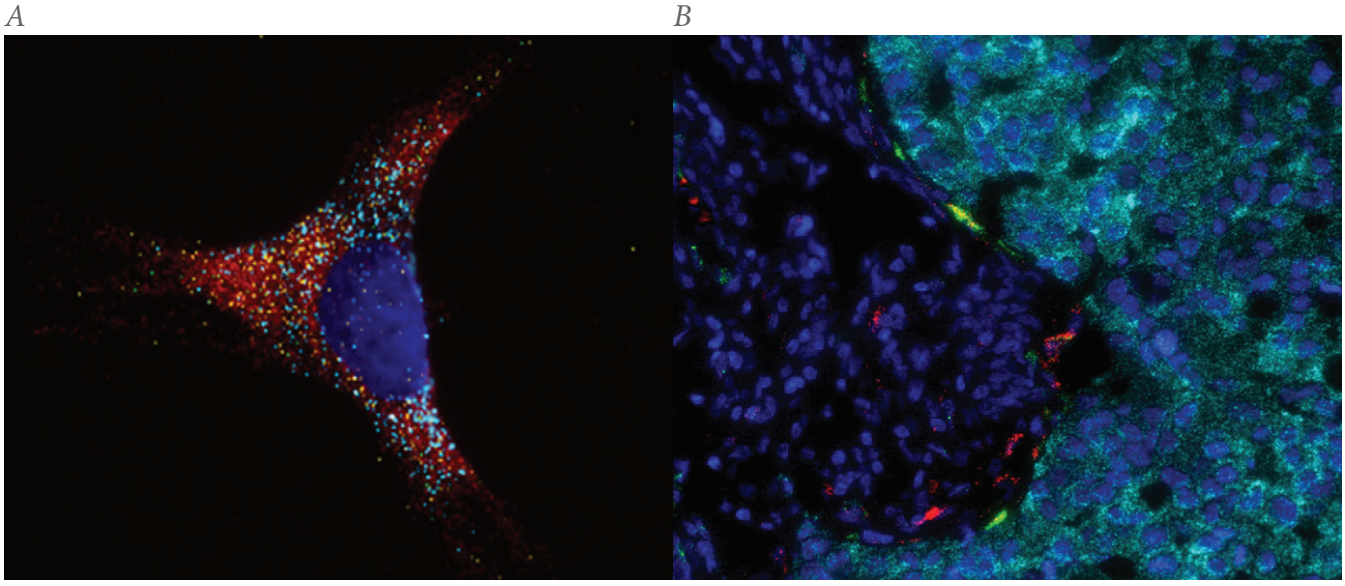

Figure 3. A. HeLa cell hybridized with probes to β-actin, hypoxanthine phosphoribosyltransferase 1 (HPRT-1), 60S acidic ribosomal protein P0 (RPLP0), and peptidylprolylisomerase B (PPIB) in multiplex fluorescence format. The nucleus is counterstained with DAPI. B. Multiplex fluorescence detection of uPA and PAI mRNAs in breast cancer. Merged pseudo-colored image of a metastatic breast cancer tissue section stained with probes specific to cytokeratins [PanCK (CK-8, CK-18, and CK-19), aqua], uPA (red), and PAI-1 (green) (11).

specimens like FFPE tissue. Adopting these techniques on commonly used automated slide staining instruments minimizes manual labor and variability, facilitating seamless integration of RNA biomarker assays into existing pathology lab workflows. The time has come to unlock the full potential of RNA biomarkers.

Because it's central to the flow of genetic information in the cell, RNA is ideally positioned to reflect cellular physiology, making it an ideal biomarker. The limitations of DNA and protein surrogates, and of analyzing RNA in solution, make it clear that there is no substitute for measuring RNA itself *in situ* – and thereby providing a direct path from the lab to the clinic. While NGS approaches continue to fuel RNA biomarker discoveries, quantitative RNA biomarker analysis that includes tissue morphology at single-cell resolution, will facilitate rapid validation of novel RNA biomarkers and help us translate them into clinical use.

Xiao-Jun Ma is chief scientific officer at Advanced Cell Diagnostics, Hayward, CA, USA.

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A Closer Look at Microscopy

No other device has captured pathologists' hearts as much as the simple light microscope – but how can hospitals and lab medicine professionals keep up with the demands of modern microscopy?

By Jan Barghaan

In 1939, Adrianus Pijper described the microscope as "man's noblest, supreme, and most far-reaching tool." Though this opinion is now three-quarters of a century old, pathologists who use microscopes every day in their work are hard-pressed to find fault with it. But in most laboratories, the microscope that sees the most use is a simple light microscope, not so different to the ones that were state-of-the-art when Pijper made his claim. So if the microscopes themselves aren't changing significantly, what is – and how can pathologists keep up?

At a Glance

- *Unlike in research laboratories, the optics for clinical microscopy don't need to be improved – but that doesn't mean the technology isn't evolving*
- *The inexorable march toward digitization means that labs are scrambling not to obtain the perfect image, but to store it*
- *In order to fully adopt digital pathology, hospitals need to commit to putting the necessary IT infrastructure in place*
- *Lab medicine professionals also play a key role in adopting new technologies, and keeping up with the times requires keeping an open mind*

A continual evolution

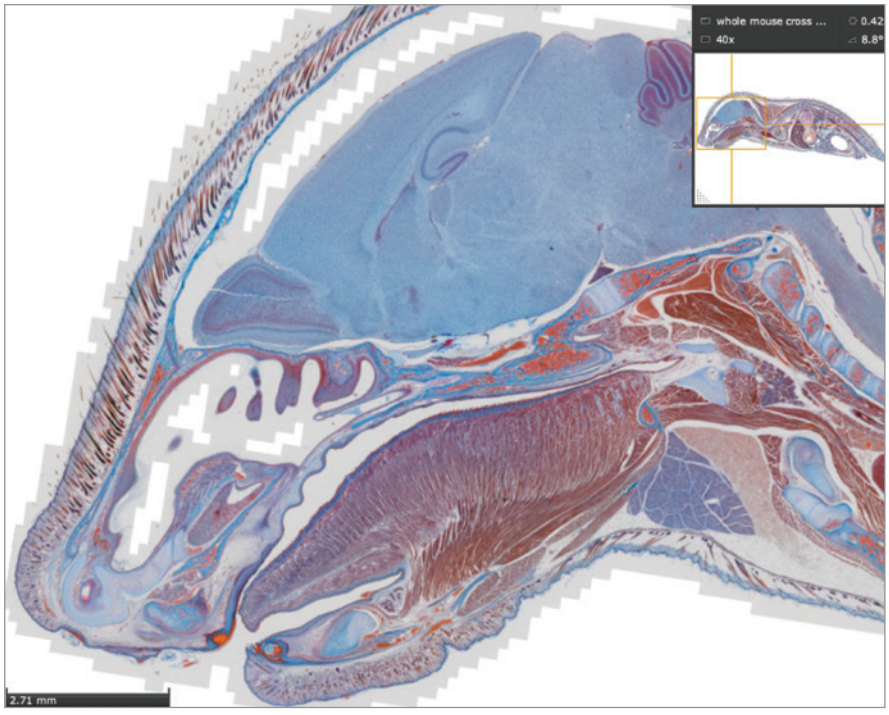
To understand how the science of microscopy is growing and changing, it's important to differentiate between research and clinical settings. Research is where new applications and methods are being developed, whereas in routine clinical microscopy, the optics don't really need much development – they're already good enough for applications like histology. There are still improvements to be made to usability, workflow, automation and ergonomics, but the technology itself works well for clinical pathology in its current form.

But that doesn't mean there aren't ways for established technology to evolve. One example of innovation in a mature technology is fluorescence-lifetime imaging microscopy. FLIM was developed about 25 years ago for applications in cell biology. It works on the basis that a fluorescence signal has a lifetime, which is usually very short but when you have a modulated light source (like an LED or laser), you can analyze the phase shift between the excitation light and the emission signal to distinguish between the lifetimes of two different signals. That lets you differentiate between two fluorophores more accurately than with standard filter-based techniques. With the recent development of easy to use FLIM solutions, this technique might become interesting for routine use. For histology slides, rather than looking at fluorescence markers, you can observe autofluorescence properties that vary depending on cell type and even subcellular structures – meaning that FLIM might allow you to see certain tissue features easily and efficiently, without the need for staining. The technology isn't quite ready for routine microscopy use yet; it's still in the process of evolving from a complicated research setup into an easy-to-use system – but I believe it's worth keeping an eye on.

It goes without saying, that the ergonomics of a microscope are of utmost importance to lab professionals, who spend long hours each day examining microscopy samples. To that end, a lot of innovation is taking place in this area, with new technologies aiming to make the user experience as comfortable and as practical as possible. Development is also being focused on making workflow better, reducing repetitive steps and automating the microscope in a way that the user doesn't need to perform so many steps – for example with slide scanning systems. I think there is still some room for improvement in that respect.

> *"No matter how you look at it, digitization results in a lot of data and data management."*

Digitization is another area of development with a huge impact on the pathologist's workflow. There's a significant need for digital slides to aid with clinical diagnosis, consulting with colleagues, and education. The big hurdle is how we handle all the data. If a pathology department goes completely digital, every slide needs to be scanned and made digitally available. Some labs even discard the original glass slide, so the digital slides need to be stored and archived indefinitely as reference for the diagnosis. No matter how you look at it, digitization results in a lot of data and data management. Fortunately, that's an area that seems to be improving in leaps and bounds!



Whole Mouse Cross Section. Digital slide scanning at 40x magnification enables virtual zooming of specific features at points of interest across the entire specimen. With a total size of approximately 2.4 billion pixels, image storage and processing pose a challenge. Credit: Olympus Soft Imaging Solutions.

Obstacles to adoption

One challenge of developing new microscopy technology is that there's still a long way to go if you want to use it for clinical diagnosis – even if a new technique is already at the point where it delivers results at a single push of a button. Before you can use it for patient testing, there have to be clinical studies to prove that the new technique can produce a reliable diagnosis. That uses a lot of resources and time, so it can be difficult. And once it's done, commercializing the technique isn't easy, either. Not only does it have to be simple to use, but you also need to make sure the new method is widely applicable in order to attract a broad user base. Without market potential, it's hard to convince a company to invest in developing your system.

But even after a technology is ready for the clinic, there are hurdles to overcome to ensure its success. With the ongoing trend toward digitizing slides, for instance, those digital images need to be integrated into the existing IT infrastructure. And again, for that, you need to be sure there's enough storage available. Big pathology departments can deal with several hundred slides per day, all of which need to be backed up, so you can imagine how fast the data accumulates – especially when you consider that many pathologists are legally required to archive digital slides for many years. Slide scanners are well evolved by this point, but individual institutions still need to take that last step and provide the IT infrastructure for them.

Last but not least, you have to win over the people who will be using your new technology. If a microscopist has spent 20 years doing their job a certain way and someone comes along and tells them to do it differently, the transition can be difficult – and requires a compelling reason to change. I personally believe that the traditional microscope will never vanish. I hear from microscope users who tell me it's a different story to see an image directly through the microscope than to see it on a screen, even acknowledging the potential benefits of digital imaging.

> *"The transition can be difficult – and requires a compelling reason to change."*

The best of both worlds

One of the most important things for pathologists to remember as technology evolves around them is to keep an open mind, and pay attention to what's going on in the world of microscopy. While digital slide technology has its obstacles, it also presents a great opportunity for laboratory medicine professionals. Usually, you have people who are either experts in the microscopes and their applications, or experts in computing and IT. There aren't very many people who are at home in both worlds, which means there's a gap that people working in laboratory science are well-placed to fill. If institutions have access to people with a background in both IT and laboratory medicine, then they really have the best of both worlds.

Jan Barghaan is senior market specialist, Life Science EMEA, Scientific Solutions Division, Olympus Europa SE, Hamburg, Germany.

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NextGen

Research advances
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SMILE, It's Easy

"Secretary-mimicking" artificial intelligence could help lift the burden of tedious report preparation

By Jay J. Ye

As a dermatopathologist and general pathologist in a busy practice, I used to devote roughly half of my time to preparing reports. But the situation has changed dramatically since Ms. Smile became my assistant two years ago. Incredibly capable, she types 1,000 words per minute, and has an eidetic memory – she forgets nothing. She can read case-associated information and preliminary reports in seconds. She is attentive to detail, frequently catching errors in section codes and specimen dimensions, as well as transcription errors in clinical histories. When I dictate diagnoses in multi-specimen cases, she knows which specimen the diagnosis belongs to without the need to tell her explicitly. Some specimens require either no slide viewing (i.e., gross examination only specimens) or only confirmatory slide viewing (e.g. when diagnoses are almost always the same, such as with vasectomy or tubal ligation), and Ms. Smile will type the diagnoses for these without being told specifically what to type. Most of the time, I agree, and she finalizes the case electronically.

With the help of Ms. Smile, my report preparation time has been reduced by around 80 percent. With her vigilance, errors have decreased, too. You're probably thinking "no way! It's not humanly possible to read and transcribe so quickly, and know at all times which slide you're viewing", and you're right. Because Ms. SMILE (Secretary-Mimicking Artificial Intelligence) isn't human…

Prelude to a SMILE

Why did I see the need to develop an artificial intelligence (AI) approach to reports? Traditionally, report preparation is assisted by secretaries transcribing what the pathologist dictates. More recently, voice recognition (VR) technology has started to gain popularity (1–4) and replace human transcriptionists. This tends to decrease turnaround time and reduce staff costs. But VR technology has drawbacks in my view: secretarial tasks are shifted to the pathologist, taking up our time, and imperfect VR systems can lead to a potential increase in errors.

Along with VR, barcode scanning technology has become more widely available. These systems track the specimens from arrival, to placement in processor cassettes, to embedding in paraffin blocks, and finally to microscope slide production. Scanning the barcode of a glass slide in a new case brings the case information up on the screen.

Prior to the creation of SMILE, I used Dragon voice recognition and barcode scanning, driven by voice activated Windows automation scripts written by a colleague of mine. It was a remarkable system, which rivals any commercially available VR technology in my opinion, and was better than traditional human transcription. But despite these improvements, I was frustrated with the fact that for many straightforward cases, I was spending more time preparing reports than I was rendering interpretations. Additionally, some reports were finalized with nonsensical errors caused by imperfect VR – it was against this backdrop that SMILE was created.

The architecture of intelligence

The underlying implementation of SMILE is a collection of over 20 thousand lines of code, and the associated data files. In order to be intelligent, SMILE has to have knowledge. This knowledge resides in both short- and long-term memory. Short-term memory contains the slide-, specimen-, and case-level information; SMILE obtains that information via non-auditory means by reading the information while the pathologist is reviewing the case. This information is short-lived, as it changes from slide to slide, specimen to specimen, and case to case. The core components of the long-term memory are integral parts of the computer program; the user-specific long-term memory resides in the data files. The voice commands are used in the context of this both timely (short-term) and timeless (long-term) knowledge, and in a logical fashion. This is the foundation of SMILE's intelligence, and allows SMILE to type reports and check for errors, communicating with the user via text to speech and on-screen messages (Figure 1).

This use of both long- and short-term memory allows SMILE to modify and even refuse some voice commands given by the pathologist – for example, SMILE will not allow a case to be signed out using the command "release case" if all the slides have not been reviewed, or the gross description contains a section code designation error. The reason for refusal will then be conveyed by voice. User input via dialog boxes continues to increase the long-term memory of SMILE, allowing it to become both smarter, and more user-specific, as time goes on.

At a Glance

- *Report preparation is important, but consumes a significant amount of the pathologist's time and effort*
- *Secretary-mimicking artificial intelligence (SMILE) can help prepare reports quickly and precisely*
- *SMILE has helped me to both improve efficiency and reduce errors*
- *Instead of focusing on voice recognition technology, we should be welcoming artificial intelligence into our daily practice*

Pathologist-SMILE interaction

Using its inbuilt knowledge, SMILE can respond to both verbal and non-verbal (slide scanning) actions by the user (Figure 2). It should also be noted that while non-verbal actions are generally not skippable, some verbal actions can be skipped (or, to be more accurate, combined into the underlying implementation) in simple cases, such as "begin dictation" and "release case", which can further save time.

To better explain how SMILE functions, I will use the Microsoft Word rendition of a series of screenshots from a real case (Figure 3). After reviewing the entire case in this example, I scanned the barcode of the slide of specimen #1, and dictated "chronic calculous cholecystitis" (Figure 3A). SMILE opened up a Word document and typed the headers for both specimens, as well as the diagnosis for the first specimen (Figure 3B). I then scanned the slide from the second specimen. SMILE judiciously put a period at the end of the diagnosis for the first specimen and then moved the cursor down, ready for dictation of the second specimen (Figure 3C). Once I had dictated the second diagnosis, the case was ready to be signed out electronically using a voice command (Figure 3D).

While working on this case, SMILE assigned the procedures (laparoscopic cholecystectomy and excision) correctly to the specimens, and for #2, SMILE changed "R lower eyelid BBC" to "Skin, right lower eyelid" before assigning the procedure "excision" to the specimen. No navigational command such as "next field" was needed, and I could also have scanned specimen #2 first and dictated the diagnosis for that specimen first, without navigational commands being required. This means the only thing I had to focus on was the interpretation – there was no need to touch the keyboard or the mouse throughout the entire process.

Functioning at an even higher level

In addition to performing in a prototypical fashion as described above, SMILE can execute many higher level requests; "understanding" the intent of the user and performing linked and complex actions, and making report preparation even more effortless.

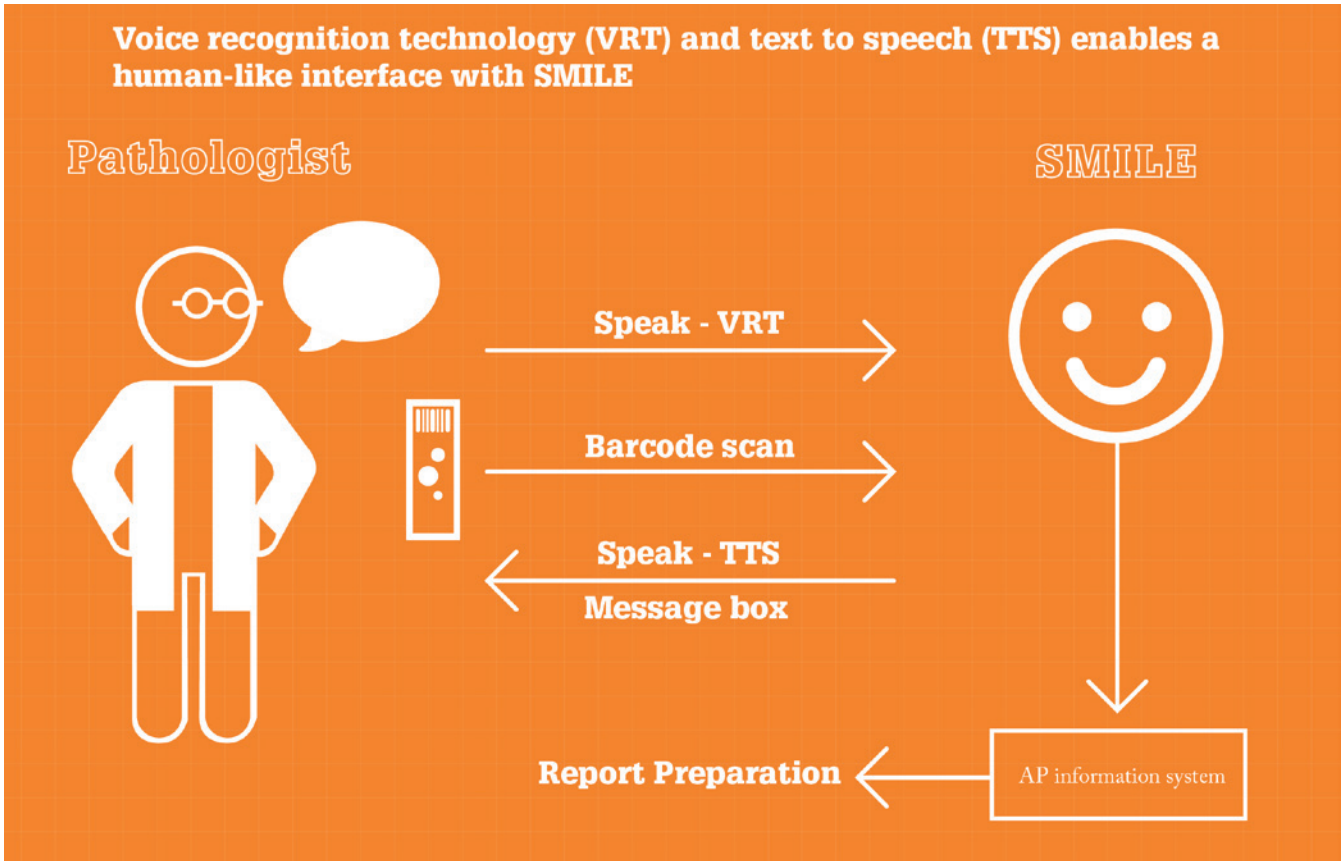

Figure 1. Voice recognition technology (VRT) and text to speech (TTS) enables a human-like interface with SMILE.

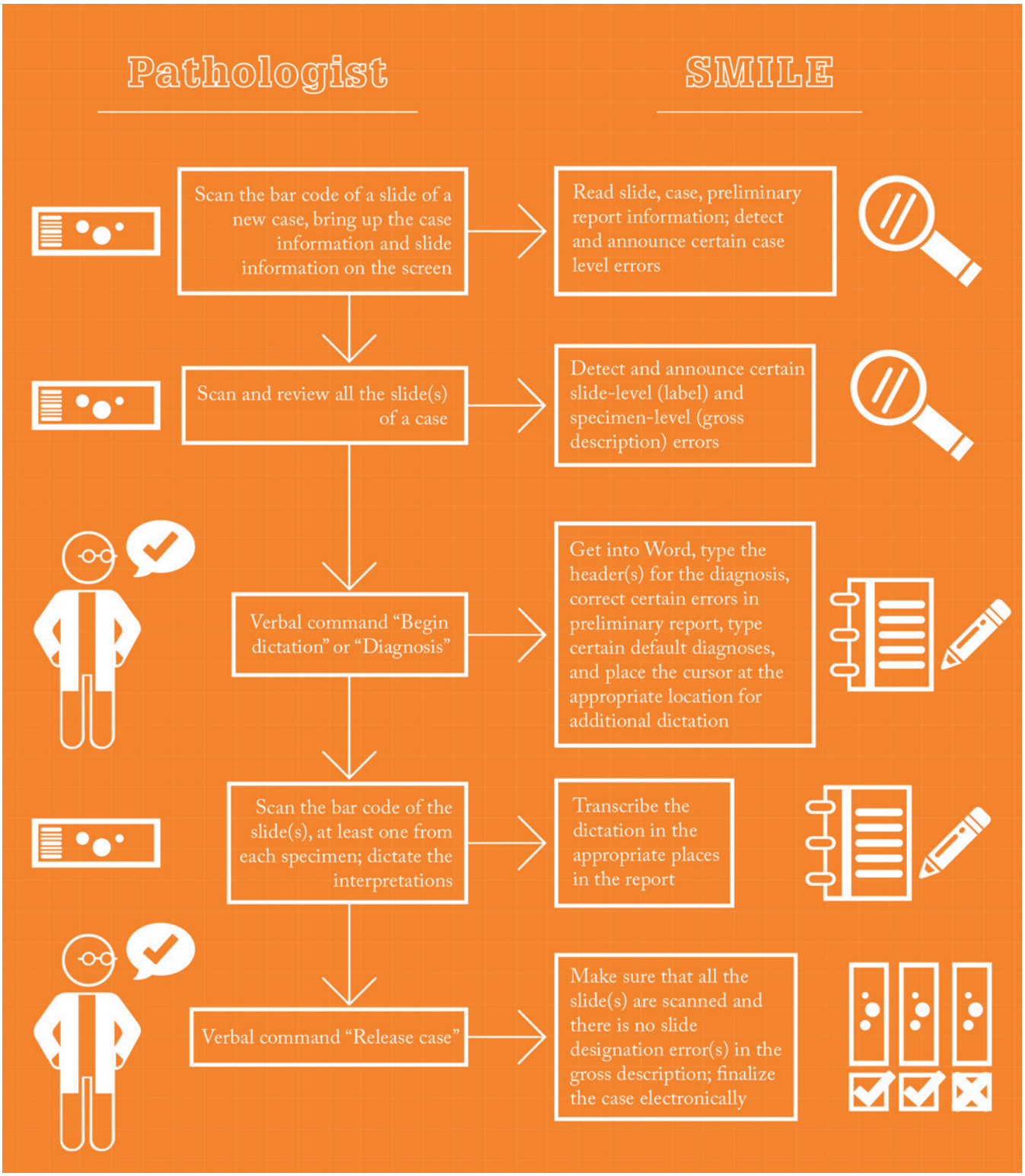

Figure 2. Pathologist-SMILE interactions when preparing and finalizing a pathology report.

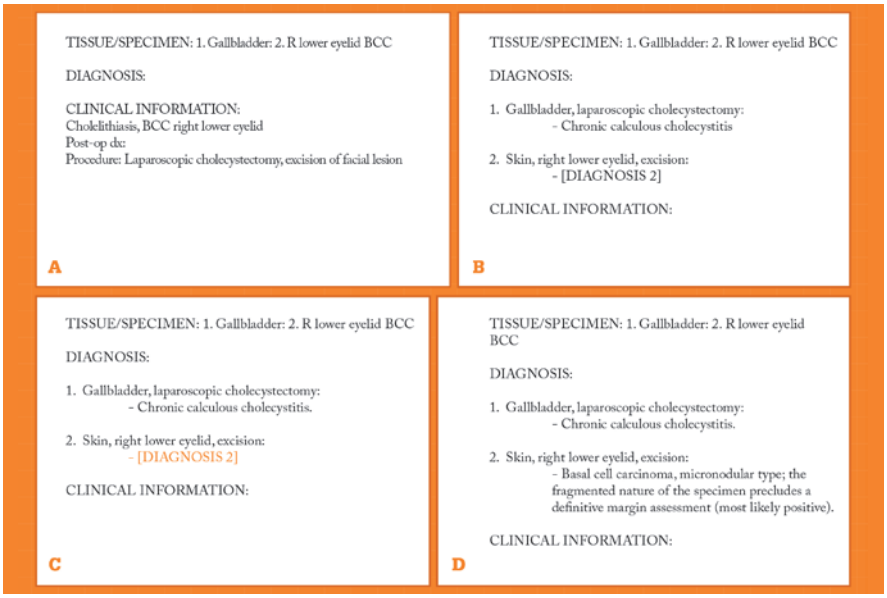

Figure 3. Report texts adapted from a series of screenshots from a real case report created using SMILE.

For example, if I have a skin shave biopsy containing superficial type basal cell carcinoma, I would say "release case superficial BCC". SMILE would type "Basal cell carcinoma, superficial type", with an appropriate header such as "skin, right cheek, shave biopsy", and electronically sign out the case. These actions are completed in 11 seconds following my voice command. If the case contained slides from two blocks, and I had reviewed only one, SMILE would type up the case, but instead of finalizing it, would tell me that a slide had not been reviewed. If there are certain mistakes in the clinical information or gross description, these would be automatically corrected for me: such as changing "seborrheic hyperplasia" to "sebaceous hyperplasia", or adding the missing unit (cm) to a gross measurement.

SMILE can further simplify the report preparation task in cases with multiple specimens. Perhaps I have a five specimen GI biopsy case – the first three specimens contain tubular adenomas, while the last two contain hyperplastic polyps. I would be able to scan the barcode of the slide of the first specimen, then say "tubular adenoma times three", and the diagnosis for the first three specimens will be typed within seconds. Scanning the slide of the fourth specimen and instructing SMILE "hyperplastic polyp times two" means the entire case is typed. Similarly, in a prostate biopsy case where every specimen is benign, SMILE only requires me to say "benign prostatic tissue times six" to transcribe the entire report.

These are just a few examples of the many ways in which SMILE can work with the user to make reporting simple, accurate and fast – a more complete description of the system has been published in the July issue of the Archives of Pathology and Laboratory Medicine (5).

Expanding AI

I believe that the introduction of SMILE to pathology practice is a quantum leap; changing from a VR-centered, pathologist-controlled process of report writing, to an AI-centered, collaborative process between human intelligence and artificial intelligence. This would allow us to focus on the tasks that require a pathologist, and reduce our time spent on the tasks that don't.

I have spent two years developing SMILE to assist me in creating pathology reports, and have been thoroughly impressed with the results – I can't imagine going back to working without it. The most important aspect of SMILE is the underlying principle it represents: it removes the mundane secretarial tasks, and lets pathologists be pathologists.

Many challenges lie ahead; the first is the current lack of awareness of both the power and the feasibility of AI for this particular niche. The second is that commercially available technology is yet to be developed – it is probably unrealistic to expect every practice to have passionate pathologist programmers ready to write their own SMILE, but both pathology information system vendors and standalone entities can potentially make this technology available. The final issue is that, with past experience of human transcription as a basis for comparison, many users are probably happy with VR systems (1, 2). But despite these considerations, I believe Ms. SMILE could soon be joining a pathology practice near you.

Jay J. Ye is a dermatopathologist at Dahl-Chase Pathology Associates, Bangor, Maine, USA.

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The Changing Face of Pathology

Han van Krieken discusses the ways in which laboratory medicine is evolving, the difference between old and young pathologists, and the obstacles facing both of them today

By Michael Schubert

Laboratory medicine is in a state of flux right now. Not only are the tools needed to do the job evolving rapidly as technology advances, but the methods themselves and even the basic skills pathologists need are changing. Whereas once a pathologist might have spent an entire career behind the microscope looking at samples from patients in a single hospital, nowadays he or she must leave the laboratory and interact with the entire patient care team to ensure the best possible treatment for the patient – and, even when seated at the microscope, might be digitally viewing samples from across the world. But as pathology changes, are pathologists themselves keeping up?

At a Glance

- *The field of pathology faces many challenges, including reduced numbers of new recruits and financial constraints, especially in the new era of molecular testing*
- *Not all pathologists are eager to adopt new technologies and new ways of performing tests*
- *It's important to establish collaborations between lab professionals, manufacturers, physicians, and the pharmaceutical industry to further new test development and to ensure payment for a drug includes all necessary testing*
- *Encouraging digital pathology and bringing in young pathologists for whom the new methods come naturally will support specialism and much-needed evolution of the profession*

Overcoming the obstacles

There are a few challenges facing pathology at the moment. One of the most significant is convincing enough talented young people to enter the field – though, according to many senior pathologists, that seems to be improving. Han van Krieken, president of the European Society of Pathology (ESP), himself says, "I hear from colleagues in many countries that pathology is becoming the center of attention, attracting more people who are really interested in pursuing a lab-based career. It's very important to encourage as much of that as possible, so that we are continually bringing in new perspectives into our profession."

The other big challenge is finance. Pathology has always been viewed as extremely inexpensive – the tests have had excellent value for money, especially when compared with things like complex imaging techniques. But now that labs have begun to move more heavily into molecular testing, doctors and patients are starting to notice that the tests cost more than they used to. And it's true that some are – a test that costs €1,000 is considered extremely expensive. But when that test is used to determine whether or not to administer drugs that cost €100,000 a year, the testing cost is quite negligible in comparison. Nonetheless, it can still be difficult to overcome the general feeling that pathology is becoming more expensive. van Krieken says that, though many pathologists do try to accomplish testing as cheaply as possible, sometimes it's necessary to pay for quality.

"To some pathologists," he says, "especially those educated in traditional methods, molecular biology can seem like a bit of a black hole. There are a lot of pathologists who are very interested and keen to interact with experts in molecular biology – but there's also a group that is still a bit afraid of the dark, so to speak. They see so many new things happening at once that they feel overwhelmed. So I'd say there's some work to be done in changing the mindset of those pathologists."

> *"To some pathologists, especially those educated in traditional methods, molecular biology can seem like a bit of a black hole."*

Cross-discipline collaboration

But lab medicine professionals aren't the only ones who still harbor reservations – the pharmaceutical industry, too, needs to be convinced. van Krieken uses lung cancer as an example of this, because there are tests that can revolutionize treatment of the disease – but only for a minority of patients. "What happens is that a patient's tumor tissue will be tested, but most patients will not benefit from the results because they do not have the genetic alteration needed to

respond well to the drug in question." In many European countries, patients have to pay for that testing, which puts them in an unfortunate position when they've paid, but can't benefit from the result. van Krieken's solution is to move toward a system in which test and drug are integrated, so that payment for the drug includes all of the necessary testing. "I know that people in the pharmaceutical industry would be very interested in such an approach," says van Krieken, "because they feel very strongly that an optimal test is crucial not just for patients, but for the industry as well, because their drugs will only work if administered to the right patients."

For that reason, he considers it extremely important for *in vitro* diagnostics manufacturers, pathologists, and medical doctors to work closely with the pharmaceutical industry to try to effect change. That level of collaboration is an adjustment for many pathologists, so van Krieken and the ESP are working to alter the way laboratory professionals approach their work. So far, they've established an educational program, started a quality assessment program for molecular testing, and built alliances with medical oncologists, gastroenterologists, gynecologists and urologists. On the industry side, they are bridging the gap between the lab, the clinic, and the pharmaceutical industry, as well as helping patients and medical professionals to influence policymakers. But even then, says van Krieken, it's not enough. "We need to engage more," he says, explaining that the ESP hired a scientific director earlier this year to do exactly that. He highlights lung cancer diagnosis as one area this level of collaboration has recently revolutionized, and anticipates an upcoming change to brain tumor classification that will also include molecular testing going forward. With these changes and others, van Krieken is hopeful that pathologists will see the benefits of reaching out and join in to help make changes to the way molecular testing is done – and the way it's paid for.

Pushing for progress

Not all of Europe is on the same page with regard to pathology. In some countries, laboratory medicine is very well-positioned – it's taken seriously, has sufficient personnel, and is an appealing career for medical trainees. "In the Netherlands, we're lucky enough to have that situation," says van Krieken, "but

there are other countries, such as Greece, that have difficulties – pathologists don't get the recognition they deserve, young people don't want to enter the field, and the science has not evolved into what we now call modern pathology." ESP, he says, actively tries to support colleagues from those countries by providing them with good education through the European School of Pathology, which holds training courses all over Europe to bring the knowledge where it's most needed. "We try to bring in those countries where pathology hasn't yet reached the position we think it should have, and help them to raise its profile."

For progress like that, ESP members are vital. "We can only help with progress once we have a foothold," says van Krieken. "We need a person, or hopefully several people, who are willing to take up the challenge of advancing pathology in their country. Then we can support those people by bringing in teachers and running courses. That's the way we try to work."

"There are other countries, such as Greece, that have difficulties – pathologists don't get the recognition they deserve."

The rise of digital pathology

"For a long time, I've felt that the era of the general pathologist who knows everything is over," says van Krieken. "Even areas that used to be fairly simple have become more complicated in the sense that we can gather much more information about our patients and make precise, accurate diagnoses." This, he says, is thanks to digital pathology. But even here, change is slow. "Pathology is quite conservative, he says, "and I do understand that, because what we do is built on the experience of our forefathers. Change is always difficult. And I think that when we looking through a microscope, we feel secure, because we've done it for so many years. Looking at the screen is a bit different – including for me. I've done a few hundred cases digitally myself, but I still feel more comfortable behind my microscope because I've been doing it for the past 30 years. Fortunately, I think it will be easier for younger pathologists."

Speaking of the benefits of digital technology, van Krieken uses the example of an organ transplant patient who wanted to have a necessary biopsy done in his hospital. "I have no experience with transplant pathology at all," he says, "so I needed the help of a colleague. When I received the slide, I just sent a link to two colleagues, one in the north of the Netherlands and one in the west – and I had an expert opinion for the patient within an hour." Another thing he notes is that his patients are becoming more and more aware of how important a correct diagnosis is to their health. "More and more patients actually send me emails asking why I've made a particular diagnosis and whether I'm sure about it. I think that's a very good development. It challenges me to an even higher level of accuracy, which I think digital pathology will enable."

Communication critical

One thing that van Krieken is seeing in many European countries is that medical students, when choosing a specialty, are starting to look not only at which ones are the most lucrative, but also at what aspects of medicine they find most interesting. And for many of them, that's the challenge of making a diagnosis using all the new testing and technology available to them. "So as we become more visible," he says of pathology as a whole, "more people are thinking, 'Well this might be interesting for me!'"

The key to this kind of progress is to make sure that pathology is very visible in medical curricula. "We used to be very visible in the old-fashioned curricula," says van Krieken, "but we were visible as 'those people who looked at slides and came up with weird diagnoses,' and that's it. In the more modern curricula, we play an integral part in the multidisciplinary team dealing with

a patient's issues. And I think that's a much better way of showing what we actually do than demonstrating basic pathology as a separate discipline. Basic pathology is certainly important, but it's not the way we work in our routine practice anymore."

In his vision of the future, van Krieken sees the pathologist as part of a patient's "support crew." The pathologist's job will be to gather a wide variety of information – morphology, immunology, molecular data, imaging – and provide it to clinicians so that they can make the appropriate treatment decisions. That's why he thinks it will be important for pathologists to get involved with the patient care team, rather than remaining separate. "Communication is one of the most important skills a pathologist can have. That wasn't always the case; I remember that when I decided to become a pathologist, one of my teachers told me, 'You don't have a communication problem – why would you become a pathologist?' At that time, intelligent doctors who couldn't communicate with patients would go into pathology. But nowadays, communication is key."

"Change is always difficult. And I think that when we look through a microscope, we feel secure."

True to this, van Krieken says the most rewarding aspect of pathology for him is the ability to interact with so many different colleagues on so many different topics. Life as a laboratory medicine specialist is filled with variety, and it's the ability to explore that and connect his discipline to so many others that van Krieken finds appealing even after so many years. And there's still plenty to look forward to as pathology evolves. "I think we have already made progress in many ways," he says, "but it comes in small steps. And, looking back, what I think will be most rewarding in the end is the ongoing evolution of the discipline, and the way it brings people together."

Han van Krieken is president of the European Society of Pathology and chair of pathology at Radboud University Medical Center, Nijmegen, Netherlands.

The Visual Pathologist

Sitting Down With… Sharon Weiss,
Professor of Pathology and Laboratory Medicine, Emory University Hospital School of Medicine, and Trustee and former President of the ABP and USCAP, Atlanta, Georgia, USA.

You've broken new ground in soft tissue pathology, being the first to describe and characterize a number of soft tissue pathologic entities – what drew you to the field?

Two things. First, my father was a surgeon, and spoke very highly of pathology, and the close relationship between surgical pathologists and surgeons. Second, I'm a visual person, and pathology is an intrinsically visual area. So much of the work is based on recognizing patterns, and matching them with disease entities, which is something that really appealed to me.

What was it like to be the first female chief resident in pathology at Johns Hopkins University?

It was an honor to be picked as chief resident, but honestly, I didn't feel very different. I was treated well, and I never felt singled out or held up on a pedestal, I was just doing my job. Johns Hopkins was very much at the forefront of women in medicine – they've really fostered the careers of women, although we didn't have many women rising to the top ranks; it was unusual for a women to be a full professor.

What are your career highlights?

Well the first one has to be graduating from medical school! Publishing the book Soft Tissue Tumors, which I co-authored, was another one. Back in 1982 it was the first real reference book in that area, and it was very well received.

Other highlights are more recent. As I became more senior in my field, I served as President of the American Board of Pathology (ABP), and also as President of the United States and Canadian Academy of Pathology (USCAP), which was great as it put me in a position to change the field for the better. One of the major initiatives I have been involved in is creating the Maintenance of Certification examination for recertification, including picking which topics we should test and how we should test them.

How important is mentorship?

I think it's critical. In my career I've had two main mentors – firstly my father, and also Franz Enzinger with whom I wrote Soft Tissue Tumors. Having a strong mentor gives you an advantage; they can not only teach you how to be a pathologist, but help you to navigate the academic waters in other ways. And I wanted to pass on these advantages.

In my career I've trained many residents and over 25 fellows in bone and soft tissue, and many of them are now doing incredibly well on their own at major institutions. It has been such a rewarding experience.

"It's very rare that someone who takes the fellowship doesn't end up hooked. And why wouldn't they be?"

What are the biggest challenges facing pathology?

We need to give pathologists more time to be creative – it's such a big specialty, with a lot to learn. But we can't just teach students how to read slides, they need more space to do research and find ways to advance the field, not just practice in it. And that's going to come from our young faculty.

But that brings us to our other problem – there's a pipeline issue. Pathology isn't well known to the public, and we don't have a major presence in medical school curricula any longer. This means we're not attracting large numbers of the best and the brightest.

Of course, there are the problems facing medicine in general: reduced reimbursements for what we do, and increasingly being forced to do much more with less…

How can pathology be made more appealing?

We need to have a better presence: pathologists need to be involved when multidisciplinary courses are taught, we need to be part of the teaching teams.

Along with this, we need to offer students dedicated experiences. For example, here at Emory we have a post-sophomore fellowship that allows medical students to spend a year in pathology as if they were an intern. Unsurprisingly, it's very rare that someone who takes the fellowship doesn't end up hooked. And why wouldn't they be? Unlike many specialties, pathology cuts across every field. You see material from children and adults, you see chronic disease, acute disease, and tumors of all types. It's such a diverse field, and when students realize that, they see the draw.

What's exciting you right now in pathology?

Pathology has always excited me. The opportunities to consult in interesting and difficult cases, and to make the right diagnoses and help my patients, continues to engage me, which is great.

Looking forward, we are on the brink of – or perhaps it's better to say we've now arrived at – a molecular revolution. It's now possible to characterize disease in a very molecular and mechanistic way, and these have the potential to translate into refined, targeted therapies. Although I'm not doing molecular research myself, I see these advances being applied to my cases, and that's very exciting.

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