the Pathologist

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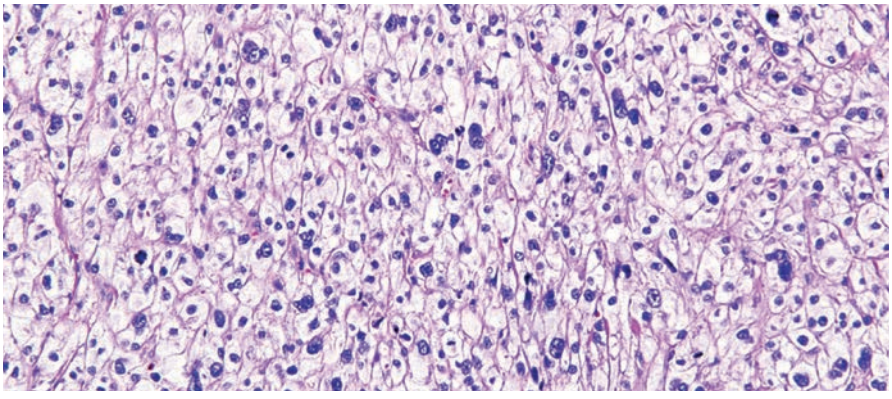
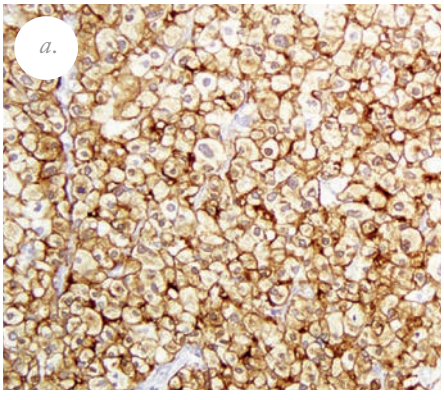
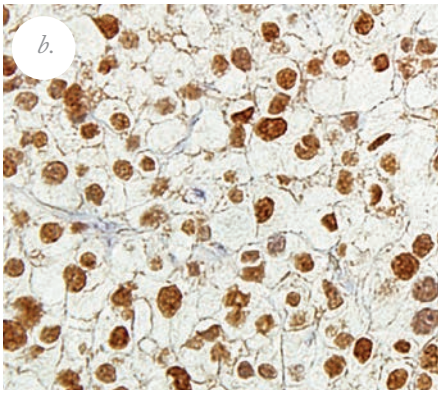
Case of the Month







Credit: Wei Cui, University of Kansas Hospital

Soft tissue tumor

The tumor shown here was resected from the soft tissue of the axilla of a 26-year-old man. The immunohistochemical stains gave the following results: positive for smooth muscle actin (a), and focally positive for microphthalmia transcription factor (b), Melan A; negative for S-100, pancytokeratin and desmin. What is the most likely diagnosis?

A Metastatic melanoma

B Clear cell sarcoma of soft tissue

C Malignant perivascular epithelioid cell tumor (PEComa)

D Leiomyosarcoma

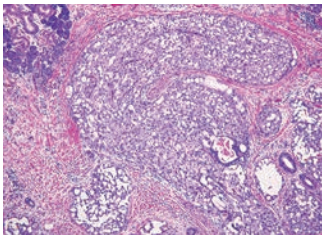
Do you think you have a good case of the month? Email it to edit@thepathologist.com

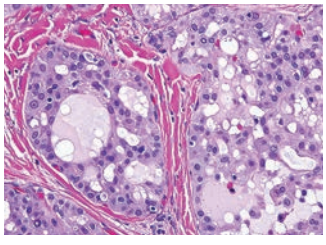
To register your guess, please go to http://tp.txp.to/0217/case-of-the-month
We will reveal the answer in next month's issue!

Answer to last month's Case of the Month…

E. Mammary analog secretory carcinoma (MASC)

This salivary gland tumor is composed of a single cell population of cells arranged into cribriform and microcystic glands, focally containing secretory material in their lumina. Tumor cells have uniform vesicular nuclei with visible nucleoli and a well-developed eosinophilic vacuolated cytoplasm. There are no mitotic figures and there is no necrosis. Overall the tumor appears histologically as a low-grade malignancy resembling secretory carcinoma of the breast (1).





Credit: Ivan Damjanov, Kansas Histopathology Club

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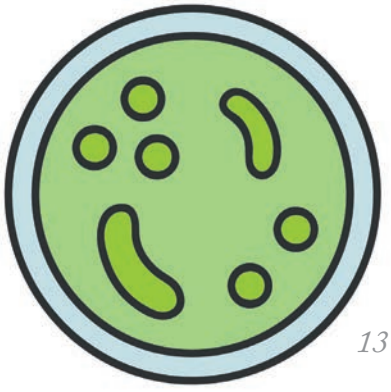

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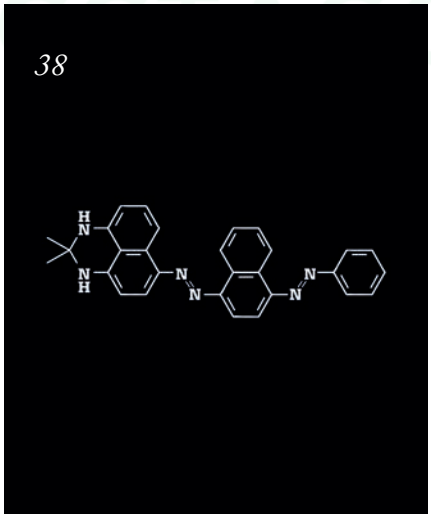

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the Pathologist

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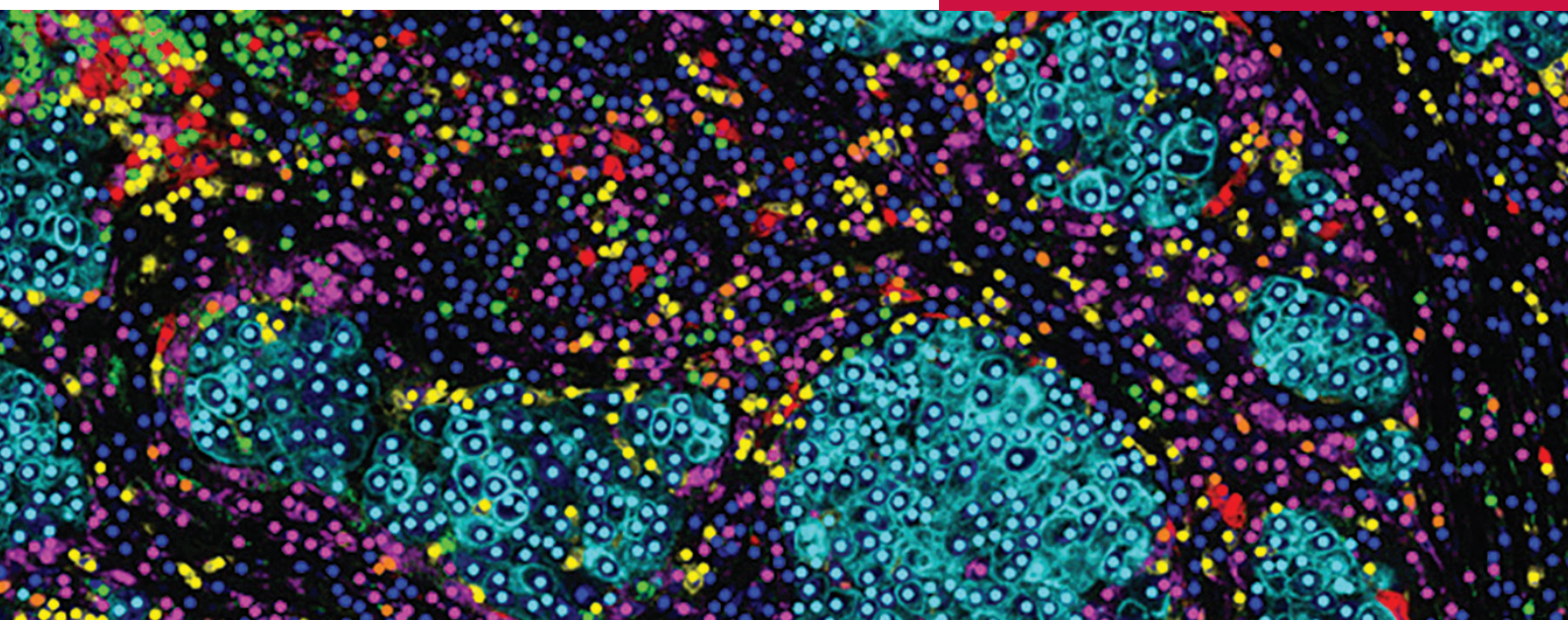

Home of the Brave

UK researchers have indicated a link between the nation's beloved sport – football – and chronic traumatic encephalopathy and dementia. Could we see a replay of US scrimmages?

In mid-February, a group of neuropathologists and neurologists from University College London (UCL) and Cardiff University published a brave piece of research (1). After following 14 retired footballers (that's soccer players to our North American readers) with dementia, they concluded that, for those "with a past history of repetitive head impacts, chronic traumatic encephalopathy (CTE) is a potential neurodegenerative cause of dementia and motor impairments." What happens when a spectator sport that's worth around 25 billion euros in Europe alone (2) is linked to CTE and dementia? Well, you can probably imagine the ensuing media storm, if you didn't witness it. You may also be able to predict the heated discussions in the boardroom of the UK's Football Association (FA) – and, although a representative for the FA publicly voiced full support of the research, aspersions were also cast...

In an interview, the daughter of one of the men, Jeff Astle (who died aged 59), said, "At the coroner's inquest, football [sic] tried to sweep his death under a carpet. They didn't want to know, they didn't want to think that football could be a killer and sadly [it] can be."

But let's add some balance. Even the report authors wouldn't call their findings definitive and issued the usual caveat: "future prospective longitudinal studies [...] are required to confirm the potential causal relationship between CTE and exposure to repetitive head impacts from playing football." However, what they did find was certainly interesting and likely not the last we'll hear of the issue.

Like me, you may be experiencing déjà vu. Perhaps the investigators would find some value in speaking with extraordinary pathologist Bennett Omalu – a man so determined to out the truth that he took on the might of the National Football League (NFL), and faced (among other things) death threats and deportation from the US. Why? Omalu discovered CTE as a result of his autopsy work on football players in the US (3) and then linked it directly with trauma induced by the sport. Omalu's relentless quest for the truth eventually led to a dramatic U-turn by the NFL – from outright denial to acceptance.

The reality is that your own work can also be subject to scrutiny. Whether a challenge to a diagnosis, a contested research result, or the rejection of your recommended therapeutic strategy – you face potential rebukes every day. Standing up for what you believe in isn't easy. But as Omalu proved, perseverance often pays.

And Dr Omalu, if you happen to be reading this, The Pathologist would love to tell your story...

Fedra Pavlou
Editor

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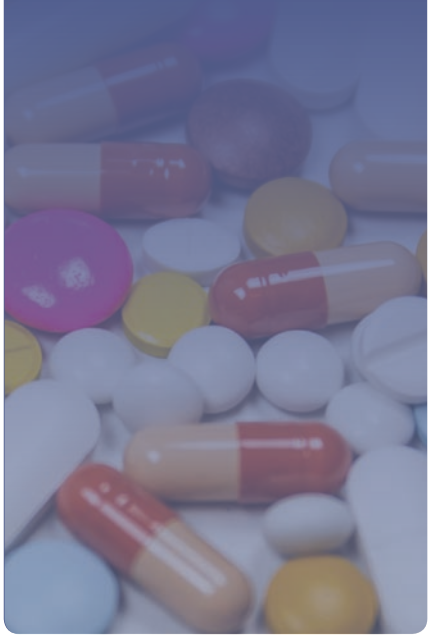
Upfront

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

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

Email: edit@thepathologist.com



Bad to the Bone

How can you tell if osteoporosis patients are taking their medicine?

It will come as no surprise to hear that low adherence to any type of medication can lead to incomplete treatment and disease recurrence. In the case of osteoporosis, patients failing to comply with their oral bisphosphonate treatment, which is prescribed to prevent a decrease in bone mass, will likely succumb to this same problem – which is concerning since a third to half of patients don't fully adhere to their medication (1).

In an attempt to curtail this low adherence, a research group from institutes across Europe and North America, in conjunction with the International Osteoporosis Foundation and European Calcified Tissue Society Working Group, has now recommended the implementation of a screening program for oral bisphosphonate adherence (2).

What might something like this – which on the face of it appears a gargantuan task – entail? They propose the measurement of two markers of the condition, levels of which are reduced by oral bisphosphonates – PINP (procollagen type 1 N-terminal propeptide) and CTX (collagen type 1 C-terminal telopeptide) – before therapy begins and then three months after its initiation. After collating their results, and referencing a study comparing three different oral bisphosphonate therapies (3), they came to recommend this: if there is a significant decrease in either biomarker (over 38 percent for PINP, and over 56 percent for CTX) then it appears that the patient is being compliant and therapy should be continued, but if there is no significant decrease, the clinician should reassess the situation to establish where the issue lies.

The detection rate of this screening – which the researchers use synonymously with sensitivity – is 84 percent for PINP, 87 percent for CTX, and 94.5 percent if both are measured and changes are found in at least one of the biomarkers. Though their guidelines are empirically based, the researchers do not suggest that their recommendations will directly affect patient adherence to medication. Detecting the issue is a step in the right direction, but finding an effective solution to low adherence is a different puzzle to solve. *WA*

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Subpar Screening

Screening for herpes in asymptomatic people may not be the best course of action after all

"The CDC estimates that almost one in six people in the US between the ages of 14 and 49 is infected with genital herpes," says Kirsten Bibbins-Domingo, Chair of the US Preventative Services Task Force (USPSTF). It's this high prevalence that drove the USPSTF recommendation on serologic screening for genital herpes in asymptomatic, pregnant women, adults, and adolescents back in 2005. But is this broad-brush approach a good one? Based on more recent evidence, the Task Force now doesn't seem to think so, and it's calling for a rethink (1).

The sexually transmitted infection is caused by subtypes of the herpes simplex virus – HSV-1 and HSV-2 – that can cause blisters, ulcers, aches, swollen glands, and fevers. However, a portion of HSV carriers appears asymptomatic, and the CDC states that most infections are spread by patients who are unaware that they have the virus (2).

"It's important to remember that any test a primary care physician does on a healthy person can potentially have both beneficial and harmful outcomes," reminds Bibbins-Domingo, so "it's important to focus on screening tests that we know, on balance, are effective." After a systematic review of the evidence, the USPSTF determined that "it was not beneficial to screen for genital herpes in adolescents and adults who have no signs or symptoms, including pregnant women – who can transmit HSV to their newborns during childbirth," she adds. Since the infection cannot be cured and test results are, at times, inaccurate, the Task Force sees the screenings of asymptomatic patients as causing more harm than good. Its paper cites that a screening of 10,000 patients would result in approximately 1,485 true-positive results and 1,445 false-positive results (1).

Bibbins-Domingo and her team now believe the current best course of action is to halt the existing asymptomatic screening method, but she encourages investigators to help find an alternative. "The Task Force is calling for more research to better understand the detection and management of genital HSV infections in people without signs or symptoms, including studies that would support the development of screening and diagnostic tests that have higher specificity and can detect both types of genital herpes infections. We are always interested in reviewing new research that can help inform future recommendations." *WA*

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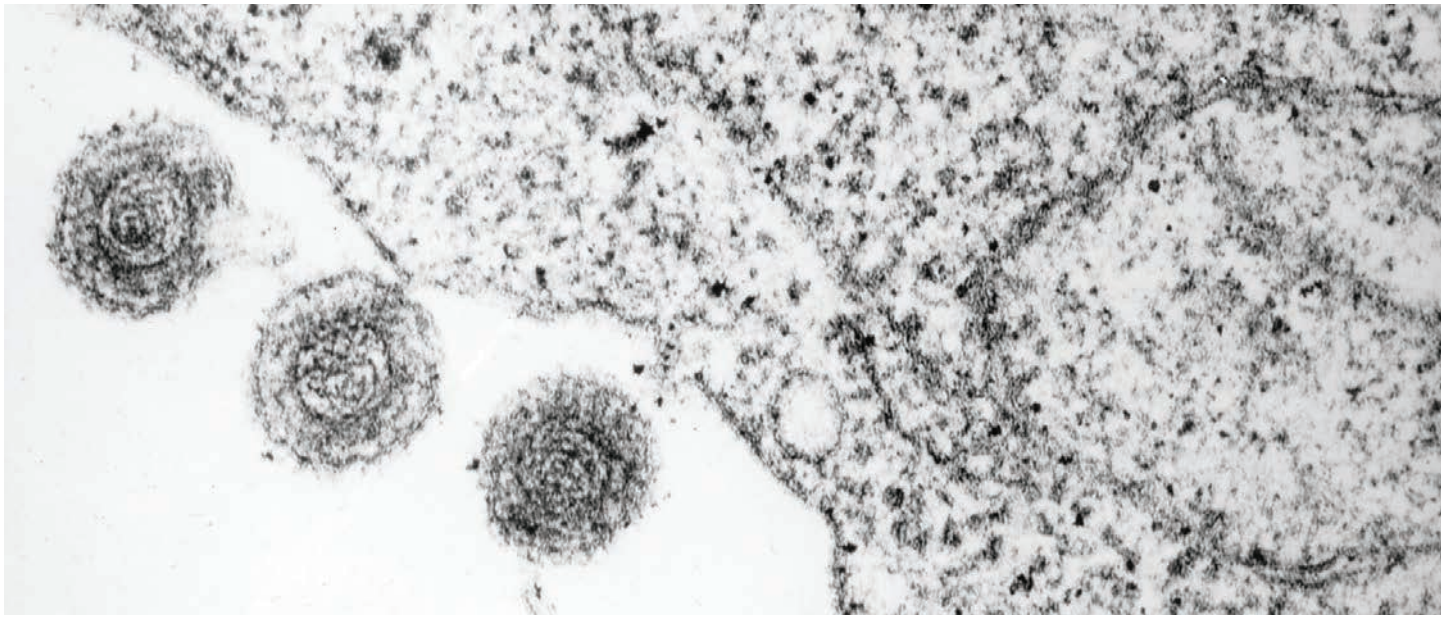

Prostate Protection

MP-MRI could prevent patients from undergoing unnecessary prostate biopsies

When it comes to prostate cancer, many agree that the prostate-specific antigen (PSA) test does not necessarily reign supreme (1). But if that's the case, what should a responsible doctor do instead to rule out the possibility of dangerous disease? One option is a transrectal ultrasound-guided (TRUS) biopsy – but this procedure is invasive and can lead to infection, or several weeks of rectal or urethral bleeding. Although the biopsy can aid in diagnosing disease, its undesirable effects and the burden it places on hospitals means it is only often used when absolutely necessary. What's the alternative? There's one potential answer: multiparametric-magnetic resonance imaging (MP-MRI), according to a team of UK-based researchers who found that the technology could be an effective triage test to help reduce unnecessary TRUS biopsies (2). To learn more about their research, we talked with first author and consultant urologist Hashim U. Ahmed.

Why did you decide to focus on MP-MRI?

There was a palpable lack of robust evidence for the use of MP-MRI in diagnosing prostate cancer. Many papers were retrospective, comparing MP-MRI with surgical specimens (which meant that all men had to have cancer on biopsy and then choose surgery), or they compared MP-MRI to TRUS biopsy (which we know is an inaccurate comparison). These methodological biases led to considerable uncertainty and skepticism about the performance of MP-MRI. This meant that practice was slow to change and there would be little traction on a wide level.

We conducted a number of studies comparing MP-MRI to template mapping biopsies, which sample the entire prostate every 5 mm and can be used in almost all men (reducing selection bias). These studies were single-center, expert academic, and all but one was retrospective. Both our studies and those conducted by other groups (3, 4) showed that MP-MRI had excellent performance characteristics, but further evidence was needed.

What do your findings mean for prostate cancer biopsies?

Patients, clinicians, and hospitals now have level I evidence to demonstrate that MP-MRI before a first biopsy improves the chances of finding significant cancer that might otherwise be missed. For those wishing to avoid a first biopsy, MP-MRI beforehand can rule out significant disease with a high degree of probability, allowing men to safely enter clinical and serum PSA monitoring.

Do you believe that imaging will play a larger role in the future of diagnostics?

We must seriously consider changing our practice across all healthcare settings to institute an imaging test – MP-MRI in this instance – before a biopsy. It is what we do for all other solid organ cancers and we now have robust evidence for a similar diagnostic pathway in prostate cancer.

What's next?

Further work is needed to evaluate the targeting of MRI areas. There are different ways of deploying the biopsy needle to suspicious areas, such as having the operator estimate the lesion's location, using devices that fuse the MRI with ultrasound, or carrying out biopsies during the MRI itself. The detection rates and cost-effectiveness

of these various approaches requires additional study.

Further work is also needed to see if liquid biomarkers could help identify men at risk before having MP-MRI, in order to reduce the costs and capacity issues that many healthcare settings may have with the new technique.

MP-MRI could also be used as a screening tool instead of PSA blood testing in high-risk populations like minority ethnicities or those with a family history of the disease. I am currently starting a study in relation to these factors.

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A Golden Opportunity?

Pancreatic cancer may have a new diagnostic, thanks to extracellular vesicles and gold nanoparticles

Extracellular vesicles (EVs) could serve as strong cancer biomarkers since the membranous sacs carry signaling factors directly from their parent cell. The difficulty comes from current diagnostic techniques – a large sample of plasma is needed to carry out an EV assay, and this can often be time-consuming, expensive, and ultimately low-throughput. But a solution to that challenge may exist… researchers from Arizona, Texas, Maryland, and Beijing, have collaborated to discover a method of detecting EVs with just 1 µl of plasma from pancreatic cancer patients (1).

Why focus on pancreatic cancer? The aggressive disease undergoes early metastasis and has a high resistance to treatment – which is why only 7.7 percent of patients survive for more than five years after diagnosis (2). This is compounded by the fact that there is currently no effective, noninvasive biomarker for pancreatic cancer.

The investigational assay requires just a single droplet of unpurified plasma, and contains gold nanoparticle spheres and rods, that adhere to cancer-derived EVs from the pancreas. The EVs with attached nanospheres and nanorods appear bright yellow when viewed under a darkfield microscope.

In addition to operating as a diagnostic, the researchers suggest that their assay could also be used to track pancreatic cancer progression and monitor therapeutic response, which could be useful since the membrane-bound EVs are much less susceptible to the degradation that conventional protein biomarkers often face.

Although the investigators believe they're a few years away from a regulatory submission, this proof-of-concept mouse model study gives hope of a future where the noninvasive diagnosis of pancreatic cancer and monitoring of therapy effectiveness might just be possible. *WA*

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Forecasting Fatalities

Could a transcriptomic signature predict the survival likelihood of Ebola patients?

The West African Ebola outbreak of 2014 was the largest epidemic of the disease in recorded history (1). Though the prevalence has declined, it remains difficult to differentiate between those diagnosed cases that may be fatal, from those that are not. In a bid to fill that gap, a team of investigators has made a discovery that may just help, by revealing that transcriptomic analysis can yield mechanisms of pathogenesis in Ebola patients (2) – information that helps produce a clearer prognosis.

"Initially, the goal was to sequence the virus in blood samples to track its evolution and to determine how this would inform both epidemiology and therapeutics," says Julian Hiscox, lead researcher and Chair in infection and global health at the University of Liverpool. "We realized the same approaches could be used to look not only at the virus, but also at what was happening inside an infected patient." To gain additional insight, the researchers looked into the transcriptomic profiles of 30,000–40,000 genes in infected patients and found that fatalities of the disease displayed a stronger upregulation of interferon signaling, whereas patients who survived showed an increased presence of natural killer cells. Transcriptomic analysis allowed the researchers to pinpoint a panel of various genes that triggered these changes and more, and used them as "strong predictors of patient outcome, independent of viral load" (2).

"Being able to look at the disease process at a molecular level provides important insights into the pathology surrounding the infection. For example, markers in the blood showing that there was significant liver damage occurring – but this cannot really be determined in a resource-poor setting," says Hiscox. That's the new technique's biggest challenge: it can't currently be carried out in the regions that needed it most during the outbreak.

Hiscox adds, for this approach to be useful going forwards, the results can't be used indiscriminately. "The data has to be taken in a wider context for future outbreaks of this nature. In this scenario, the triage of patients would allow resources to be directed to those who need it most, and will also provide a framework for how to implement placebo-controlled clinical trials in an ethical framework. For instance, to give the experimental therapy to those most in need, and the placebo to those more likely to recover."

The team continues to investigate Ebola and its role in the outcome of co-infections, while exploring more forecasting opportunities. "We are taking the predictors further and seeing whether these are specific to Ebola, or more reflective of acute febrile illness. More generally, our laboratory work has set a framework for ongoing studies of hantavirus, influenza virus, and respiratory syncytial virus," adds Hiscox. *WA*

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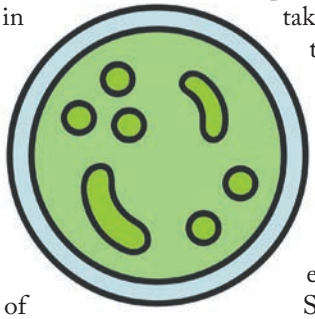
Protecting the Last Resort

Detecting antimicrobial resistance to emergency antibiotic colistin

As antimicrobial resistance persists, finding drugs that effectively fight antagonistic microorganisms becomes a more important endeavor. Often referred to as a "last-resort", the antibiotic colistin combats several multi-drug resistant bacteria – but even it isn't immune to the creeping cull of resistance. The gene *mcr-1*, found in species of gut bacteria, gives microbes the ability to resist the effect of colistin, and, worryingly, its protective traits are transmissible both vertically (via genetic fission; common resistance) and horizontally (via plasmids; mobile resistance).

Of great concern is the widespread impact that transmission might have if left unchecked. Bacteria that acquire colistin resistance through horizontal, or so-called mobile, transfer of *mcr-1* could become untreatable in humans and might also give rise to resistance in other common strains of human-borne bacteria. It goes without saying that detection of the gene is essential in order to contain its escalation – which is where a team of researchers from Germany and Austria step in.



Employing a loop-mediated isothermal amplification (LAMP) system, which uses PCR and WGS, the investigators were able to distinguish plasmid-embedded *mcr-1* from vertically-transmitted intrinsic *mcr-1* in multi-drug resistant "superbug" *Enterobacteriaceae*, with 100 percent sensitivity and specificity (1). According to the team, this is the only system to have been shown to accurately differentiate between common and mobile resistance gene transmissions, since they are phenotypically identical. The test takes approximately 20 minutes to carry out with only two minutes of hands-on sample-time needed, and the researchers reiterate that its rapid nature is vital to contain the spread of colistin resistance as effectively as possible.

So far, their findings have been taken from bacterial cultures, but the team plans to keep working on the technique to be able to safely implement it as quickly as possible. *WA*

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FLI on the Wall

A more effective metric for predicting NAFLD has been discovered

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in Western countries (1). A condition that is strongly associated with progressive hepatic disease, cardiometabolic disorders, and chronic kidney disease, it's thought to affect more than 30 percent of adults in the industrialized population, and this prevalence skyrockets to 70 percent in the morbidly obese and those with type-2 diabetes (2).

In spite of the morbidity that NAFLD inflicts, liver biopsy remains the current gold standard for its diagnosis which, unarguably, is far from ideal. In order to trim the fat and find a simpler surrogate, a group of investigators analyzed four different diagnostic markers to find a noninvasive alternative – which they identified as the widely used fatty liver index (FLI) (2). Although it was the strongest candidate in their investigation, the researchers wanted to improve upon its power, which is when they turned to the blood-borne biomarkers plasma triglyceride, and glucose and lipid levels. In conjunction with FLI and a single nucleotide polymorphism from the *PNPLA3* gene (the strongest determinant of NAFLD), they noted that the biomarkers could help calculate a more effective rubric for diagnosing NAFLD. The metric – dubbed "extended FLI" – considerably improved upon the strength of FLI to determine cases of NAFLD.

The investigators point out that there's still a lot of work to be done in order to further validate extended FLI – considering that they mention their study population only included Caucasians at risk of type-2 diabetes. But they remain hopeful that, with further testing, the technique could become a widely-used method for detecting advanced stages of NAFLD, and could even turn out to be a good predictor of fibrosis as well. *WA*

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In My View

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

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

Contact the editor sat edit@thepathologist.com

Digital is Inevitable. What Now?

Pathology is not immune to the powers of digital; let's look at parallels outside the medical industry...

By Patrick Myles, Chief Executive Officer of Huron Digital Pathology

It's amazing to think about how digital imaging technology has transformed just about every part of our lives. Want to watch an Oscar-winning film in 4K resolution? Just fire up Netflix and grab the popcorn. Need a 360-degree view of your car's surroundings so you can safely park at the grocery store? It's right there on your navigation screen. Have a desire to take magnificent photos that can be blown up and displayed on huge billboards in major cities around the world? Press a button on your iPhone (last year's model will do just fine).

This begs the question: are there really any areas of our lives that won't be disrupted by digital technology? I read with interest a recent cover feature in The Pathologist on building a business case for digital pathology, and specifically Luke Perkocha's thoughts on the issue (1).

If you read various opinion pieces on digital pathology, you may think that the practice of pathology is somehow structurally immune to the powers of digital. Is this true though, or is the march towards digital inevitable?

Perhaps the strongest argument about pathology's digital future, both for and against, is the radiology analogy. It goes like this: if you think that pathology will go digital, you will argue that digital imaging is everywhere in radiology, so therefore it's inevitable for pathology. If you are in the opposite camp, you will agree that digital radiology replaces costly and messy film; however, pathology slides aren't going away, and their digitization step is complex. Plus, if you're against it, you'll argue that digital radiology images are small and manageable, whereas digital pathology images are large and unwieldy. Unfortunately, these lines of argument quickly lead to a dead end, with entrenched parties on both sides.

How about this: let's step out of our comfort zone for a moment and look at a non-medical industry that has a strong professional component and has gone through a digital imaging transformation. Perhaps there may be some insights there. To do so, I will focus on professional photography and where it was at a similar stage in its evolution. We will see if we can answer the question of whether pathology has special digital-fighting powers or whether we've been down this road before.

Twenty years ago, we were still largely in the film age. Around that time, I remember when Philips came out with the first 35mm full frame image sensor that had the resolution (6 megapixel at the time) and dynamic range to offer up a true alternative to film in a professional setting. In 1998, Phase One was the first company to incorporate Philips' sensor into their single shot digital camera back, which attached to the back of a Hasselblad studio camera in place of the traditional negative film holder (a close analogy to putting a digital camera on a microscope). With this new technology, professional photographers could now offer their customers digital files almost immediately, rather than

waiting until the next day for negatives to be developed in the lab and for film to be scanned into digital format. Once advertising agencies got hooked on this quick turnaround, they naturally gravitated towards photographers who had these new digital tools. With some enticing financing from the vendors to purchase the expensive equipment, professional photographers began trading in their film backs for camera backs in record numbers. The rest is history.

"In pathology, many vendors count the massive number of slides to be digitized and just see dollar signs."

But surely, there must have been some photographers who resisted these new tools, right? To provide some context, I found a wonderful "for and against" article that was published by Computer Weekly magazine in 2000 titled "Will digital cameras supersede film photography?" (2). In the article, the "against" camp makes several arguments that haven't held much water. For example, the one author argues that the cost of storage is a huge impediment to adoption of digital photography. He uses the example of a then state of the art digital camera that would quickly fill up an 8-megabyte memory card (that's megabyte, not gigabyte). He also makes several interesting arguments about printing digital files – how it's too expensive to print using digital and the files don't have enough resolution. I guess not everyone could have predicted that print would be so vastly replaced by the many screens in our lives.

For pathology in 2017 – to quote baseball great, Yogi Berra – "it's like déjà vu all over again."

First, let's address storage. In 2000, 8 MB of compact flash memory was actually a big deal. It was relatively expensive, it had to be fast enough and it had to be reliable. But look what happened in three short years. In 2003, a 512 MB memory card was selling for $150! Today we see the same dynamic. For some reason, we find ourselves caught up in a terabyte dilemma when, in fact, we're entering the age of the Petabyte (get used to that word). Forbes contributor and technology guru, Kalev Leetaru, gets it right in his recent article "Why are we so afraid of petabytes?" (3). He asks why terabytes are feared in 2017, when Google and Facebook have been routinely working on multi-petabyte datasets for the past five years. Or look at retailing giant Walmart. The company is in the process of building the world's largest private cloud to process 2.5 petabytes of data per hour (4). The lesson here is that medical imaging, and just about every other part of our lives, will benefit immensely from the amazing advancements that are happening in technology-leading industries.

The other lesson from digital photography is in the misplaced belief that with digital technology we will simply be doing the same things as we did before, just somehow faster and cheaper. This belief totally ignores the power of technical innovation and its ability to create unforeseen applications. In 2000, photography was seen primarily as a means to get high quality images onto the printed page. Whether it was a newspaper, magazine, or billboard, that's where photographs went. Of course, what happened was the digital capture tools were combined with higher capacity storage, faster processors, broadband internet and all manner of amazing online technology to create incredible new products, applications, and servers – only some of which could have been imagined. The implication for pathology is that digital technology will enable new ideas and innovations, and that we must look beyond what we are doing today – and beyond our own front door – to see where the opportunities are.

Okay, so digital is inevitable. What now?

It sounds like I have been a little hard on the practitioners, whether photographers or pathologists, but let me be equally tough on vendors. They are often responsible for inhibiting adoption as well. In pathology, many vendors count the massive number of slides to be digitized and just see dollar signs. They miss that critical first principal that "great products solve problems." There's a reason that whole slide scanning tools have been embraced by the research community – the researchers have real problems to solve. They need to get their slides into a digital form to complete their quantitative research. Very practical indeed. The very same thing needs to happen on the clinical side. Instead of trying to scan every slide right out of the gate, we should start with the low-hanging fruit where digital technology can solve pressing, real-world problems. Then we can work together to see where the digital future will take us.

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Drowning in Data

Who has the right to wield the power of genomic sequencing – and what should we do with the secrets it reveals to us?

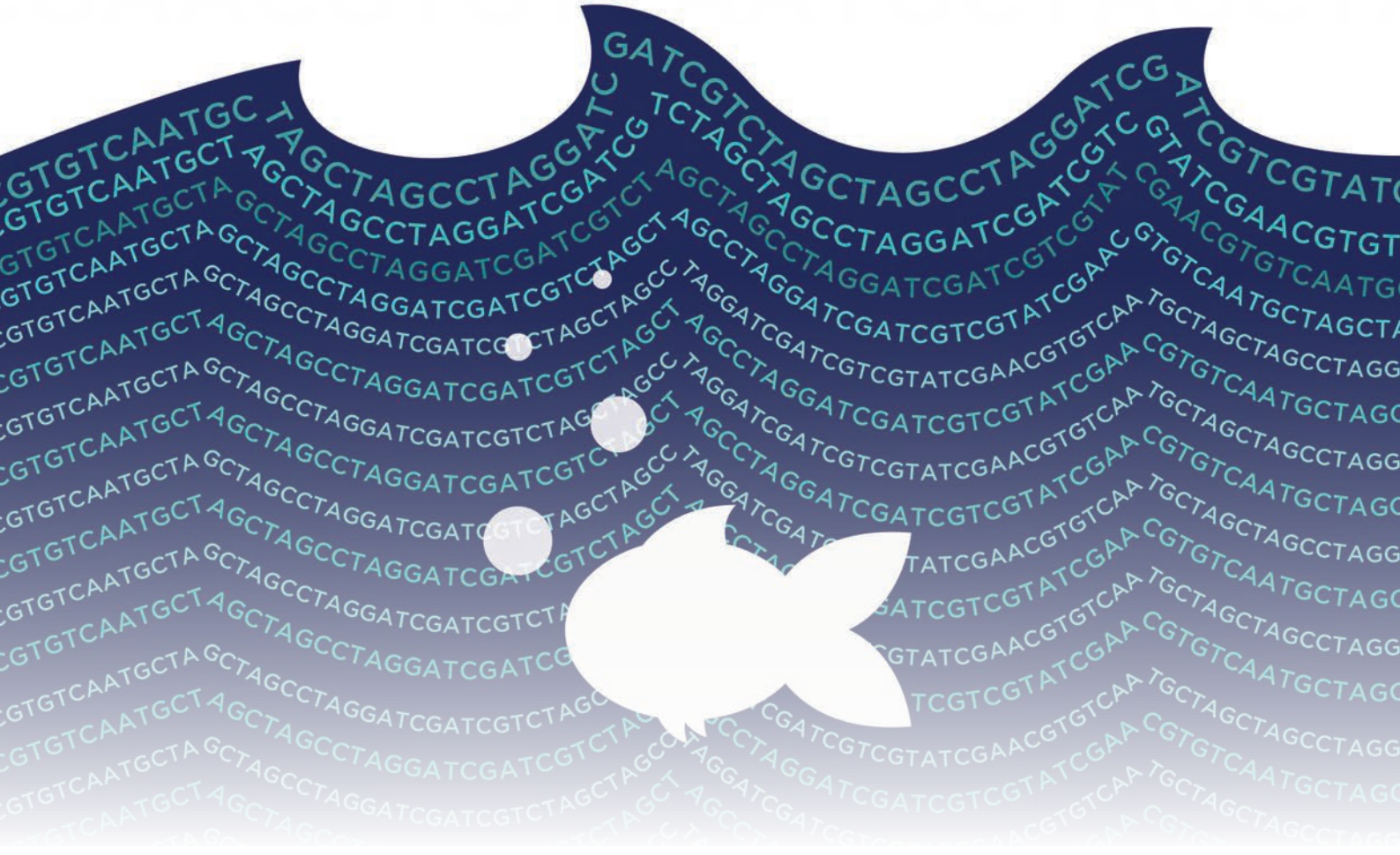
By Harriet Feilotter

Genetic analysis gives us access to a wealth of information about our patients – but are we getting too much of a good thing? The benefits of that information are obvious, but the drawbacks are somewhat more complicated. What do we do with all of this new data? How do we curate it? What parts of it do we analyze, what parts do we interpret, and what parts do we provide to the clinician or the patient? And just who has the right to make those decisions in the first place?

Why am I so concerned by these questions? I'm a molecular geneticist running a diagnostic laboratory in an academic health science center. My lab offers testing for both hereditary and somatic conditions, so we have to be quite flexible in terms of the types of assays we develop. At the same time, like most of our colleagues, we have a limited budget – so we try to restrict the number of different platforms we use to meet our patients' varied needs.

Several years ago, we began using next generation sequencing (NGS) assays. Why? It seemed clear to us that those platforms best supported the generation of large volumes of data from very small volumes of patient samples. The flexibility of the technology we use to identify relevant mutations for inherited and somatic disease alike meant that we could purchase and maintain fewer total pieces of equipment without compromising our ability to offer an appropriate range of tests.

Our small Canadian molecular laboratory is not unique. Regardless of jurisdiction, a similar revolution is taking place in almost every molecular diagnostic lab as NGS platforms move in and take up residence. For cancer testing, the appeal is obvious – labs can now generate data on tens, hundreds or even thousands of gene sequences using incredibly small amounts of patient tumor material. Even in the hereditary disease setting, the ability to obtain vast quantities of sequencing data from a



single run comes as a big relief, allowing us to replace the slow historical approach of interrogating sequential gene candidates using labor-intensive Sanger methods. In either case, NGS gives us the power to deliver much more detailed results much faster than we ever could before.

But the thrill of the high-throughput platform is tempered by an abundance of questions. Which genes should we sequence? Which variants should we report? And what should we do with this unprecedented amount of data?

Placing your order

The questions start at the very beginning, as we decide on the best approach to the assay itself. We're spoiled for choice; NGS platforms are quite accessible, because vendors have made great efforts to provide both commercially-validated panels for a multitude of uses and support for laboratory-developed tests (LDTs). But whichever type of test we want to use, our first decision must be about the extent of its power. Is the patient best served by a genome-wide interrogation? Perhaps only the coding regions of the genes? Or a set of most likely candidate genes? Perhaps a hotspot approach to assay only actionable changes? Each of these possibilities carries its own set of pros and cons, and our decisions have to be made against the backdrop of an ever-changing knowledge base. Many labs will hedge their bets and validate an assay that is not strictly confined to actionable changes, knowing that information they can't use today could easily be a drug target or diagnostic assay tomorrow. This leaves molecular labs with the tricky task of balancing their assay selection to meet both current and future needs – without necessarily even knowing what those future needs might be. And because each clinical testing lab faces different constraints in terms of budget, resources, knowledge and ability to predict the future, decisions like these fuel the lack of standardization that characterizes the current NGS era.

The use of commercial panels has an advantage over LDTs in that those assays are validated by the vendor, reducing the technical burden on the lab. The tradeoff is that the lab has no control over the panel content. So, for instance, I might be interested in looking for variants in specific areas of *KRAS*, *EGFR*, *BRAF*, *KIT* and *NRAS*, so I might select a panel that includes all these regions of interest – but also includes other cancer genes. That's certainly a viable option, but now I'm left with another quandary: what do I do with all of the extra data that the clinician didn't ask for, and the patient didn't consent to have investigated?

> "What do I do with all of the extra data that the clinician didn't ask for, and the patient didn't consent to have investigated?"

Many labs avoid this particular problem by assembling a custom panel to meet specific needs. In that case, they make decisions about which parts of which genes are clinically relevant, and then design the panel to accommodate only those targets. But those labs have to be careful, too; assays that are too focused are not very future-proof. Odds are that the lab will need to redesign and revalidate an expanded panel before much time has passed, just to keep pace with growing knowledge about the genetics of the lab's disease or disease group of interest.

Genetics test kitchen

Regardless of the selection process, the assay must be put through its paces prior to being placed onto the clinical lab test menu. Validation processes (1–4) include identifying sample types that are appropriate for testing, ensuring that the method of nucleic acid extraction is adequate, and defining the metrics of analytic sensitivity and specificity. Even so, not all targets are equal; these parameters can be highly influenced by the genomic context of a given variant, leading to the possibility that some areas are less well scrutinized than others. It's important to know where these areas are, and to determine whether or not we might

Points of View

An interview with Steven Ralston

What are the main pros and cons of genetic panel testing?

Access to genetic testing gives patients important information about their health, which may help them in making many health care decisions. On the flip side, results can be difficult to understand or interpret, not every test yields information that is actionable, and testing also has consequences for genetically-related family members.

What are the most important ethical issues to consider with this type of testing?

It is imperative that providers and patients understand the limitations of genetic testing and the possibility that results may not be unequivocally predictive of the presence (or absence) of any particular disease. A patient once said to me, "I thought that because it was a DNA test it was 100 percent accurate." I think this perfectly demonstrates one of the most common misconceptions.

I also think the question of incidental findings is crucial: what should testing companies or laboratories do with information that is gleaned from genetic testing that is unrelated to the question originally posed or the disease initially screened for?

What can health care providers do to address these issues?

We must educate ourselves and our patients about genetic tests, their indications and limitations; this is key.

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need additional measures to completely evaluate an intended set of targets. Likewise, for the foreseeable future, it remains impossible to validate every single variant we might detect in an assay with thousands of targets. So how do we manage validation? It relies, at least to some extent, on extrapolations of the panel's performance in detecting different types of mutations – be they single-nucleotide changes, small insertions or deletions, or larger structural changes. Assays continue to provide information about their performance, and labs continue to learn about their limits, long after they are placed into clinical service.

> "If we continue to adhere to this policy of validation testing as NGS panels become more routine, it's likely to have a major impact on our lab budgets."

There's also the question of orthogonal validation of important variants that might be identified in an NGS run. Many labs require that clinically relevant variants be verified by a second method appropriate to the sample type – but there is a price to pay for this comfort. First, there isn't always sufficient material to carry on to a second platform. And second, this standard isn't applied to tests across the board. If we continue to adhere to this policy of validation testing as NGS panels become more routine, it's likely to have a major impact on our lab budgets – something not every lab can afford, which is why I anticipate that we'll increasingly begin to curtail our reliance on this additional testing.

A sample's journey

When a fully validated assay is available, patient samples (like blood, plasma, tissue, saliva, urine or bone marrow) can begin to flow through the lab. For hereditary conditions, the sample is generally sent up to the laboratory along with relevant paperwork. For somatic cancer, the tumor block is usually sectioned and a stained slide examined by a pathologist, who marks the appropriate area for nucleic acid extraction and testing prior to the molecular lab's receipt of the sample.

Once in the lab, the sample information is accessioned into a secure database and the appropriate test ordered. We extract nucleic acid (usually DNA) from the sample and assess it to ensure sufficient quality and quantity. Then, we perform the assay, the technologist completes a preliminary analysis, and the results go into the secure database. Finally, the raw data go to the laboratory director or designate for final analysis and reporting. What does that entail? The lab director examines the data, compares the finding to that of the technologist, and provides a clinical interpretation of any variants identified. He or she then issues a report that explains the assay, its limitations and parameters, the result, and the clinical impact of that result.

This routine, in-and-out process – wherein patient samples come into the lab and results flow back out to the referring clinician – doesn't address the larger questions that arise when we use panels that include genes and variants not specifically requested. In an era where lab testing is so expansive but the clinical utility is restricted to a handful of genes or variants, how do we deal with the "extra" data? This leads us to an important ethical question: should complete information derived from patient samples be provided back to the patient and their care provider? And if so, how?

The good, the bad and the complicated

The ethics of using panel testing can be considered in multiple ways. On the one hand, we are generating information that has not been requested, may not have been discussed with the patient or clinician, and may be of limited or no immediate clinical value. We might identify clinically meaningful variants that are not germane to the condition at hand, or variants with dubious clinical relevance. Disclosing such results to the referring clinician and ultimately to the patient could result in increased anxiety for no particular gain. Therefore, one might argue against panel testing because of the potential harm to patients and the increased burden on clinicians to handle these results when they do occur.

The other view suggests that it would be unethical to consume small and precious samples for only a very limited number of investigations when a much broader panel could provide the same clinically relevant results and a host of additional, near clinically relevant information as well. In the case of hereditary disease, it could also be considered ineffective patient care to test only for variants in a limited number of genes, when a negative result would trigger additional testing in a broader set of gene targets anyway. And then there's the human factor to consider. It's highly unlikely that labs and scientists would agree to put the genie back in the bottle, as it were – there's no

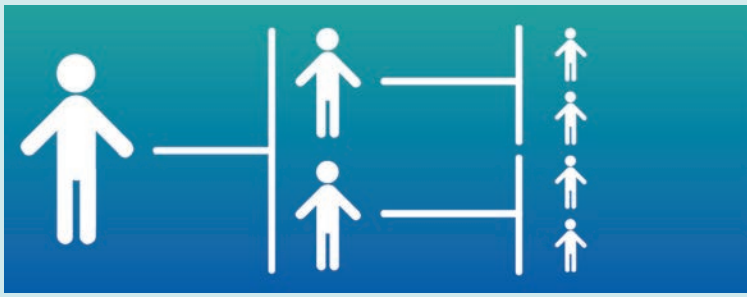
way to "unsee" the power of NGS approaches in the patient setting. Instead, we must determine thoughtful approaches to dealing with the consequences of using this technology.

What can we do? We have several options:

- Not analyzing data beyond that specifically requested.
- Analyzing all of the genes in a test, but disclosing only the specific result that was requested.
- Obtaining informed consent from patients prior to testing, ensuring they understand the scope of the test and possible outcomes.

The first option seems wasteful – why generate all those data without ever analyzing or using them? The second seems ethically questionable and leaves lab directors in the untenable position of having to make the call on what to share and what to keep back. The third option is likely the best for the time being, but it's cumbersome and requires significant buy-in from clinicians and counselors. It also requires us to thoroughly explain the complexities of panel testing, the potential outcomes, and the changing landscape to patients, who are naturally more worried about their own health and may not be focused on taking in a high volume of complex information. Nonetheless, a thoughtful approach to informing both patients and clinicians of the power and pitfalls of panel testing could at least ensure that everyone is forewarned of the possibility of unexpected results – making those conversations, if necessary, easier. This option also lets patients consent to the use of their data in research, one more way to maximize the value of very small samples.

This solution, however, brings up several issues for the clinical testing laboratory. First, who is responsible for the interpretation of the "extra" data? And how deeply must that person explore the scientific literature to be sure he or she can provide up-to-date information about variants whose clinical relevance may still be a long way off? Dealing with variants of unknown significance (VUS) is not a new problem, nor is it specific to panel testing. Any interrogation of DNA has the potential to identify a VUS, and clinical laboratories need a stated policy for handling them. Disclosing a VUS result to a clinician or patient can result in anxiety, frustration and misunderstandings – but non-disclosure can result in omitting information from the patient's record that could one day, through lookback testing (reinterpreting results in the context of new scientific findings), be clinically important. Lab directors are not – and should not be – in the business of deciding which results to pass on and which to hold



The Power of Pedigree

By Leigh Stott

I can call to mind one example when the fallibility of panel testing was really highlighted, and that was in a rural Australian mining and farming community with a higher-than-average (1 in 25) population cystic fibrosis (CF) carrier risk. A family physician referred a Caucasian couple to a genetic clinic for prenatal CF counseling. A detailed family pedigree showed CF-affected individuals in the paternal lineage – but according to the couple, the father himself had been "tested for everything, but nothing was found." Based on this lack of result, the family incorrectly believed that their pregnancy carried zero risk of CF, and that there was no need for concern or maternal testing.

Why was this such a problem? Recent research suggests that there are more than 100 disease-causing CF mutations, but common panels search for only about one-fifth of these.

After reviewing paternal panel testing documentation, a variant of unknown significance was reported. Additional counseling encouraged the mother to proceed with carrier testing – which returned positive for ΔF508, the most common CF mutation. Based on pedigree and testing, the family was advised of a potential 25 percent risk of a CF-affected pregnancy.

This case illustrates the real risk associated with patients' perception of infallibility in panel testing. Most genetic disorders don't have reliable testing available – and even in those that do, the test is not an absolute guarantee. Variants of unknown significance, unknown disease-causing mutations, and the preconceptions of non-genetic medical professionals leave significant and potentially harmful gaps. That's why I'd like all doctors to remember that the family pedigree remains the ultimate and most essential tool in genetic counseling.

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back. That means the onus is on the lab director to ensure that VUS are appropriately researched and explained, and that the data used to determinate a VUS are kept accessible. Just what that means in the context of "additional" findings from NGS panels is an important issue, and one we as medical laboratory professionals need to discuss.

A second and more controversial issue is that of follow-up. As scientific evidence accumulates, variants move from being investigational tools to being validated targets that guide clinical management decisions. Who is responsible for linking historical information about a specific patient sample with newly emerging data? No part of the health care team is currently equipped to deal with the issue of "lookback" testing. There is some guidance around this issue as it relates to hereditary variants (5), but institutions are still left to themselves to identify best practices and implement a process that works well for patients and professionals. Even efficiently tracking which samples carry which variants is a huge logistical problem, because for many molecular labs, data issues like storage, handling, annotation and linkage are still unfamiliar territory.

Finally, there is the question of ownership. Who "owns" the data generated in laboratory investigations? If patients insist on being provided with a full record of results from a genetic assay, how does the lab handle the fact that some targets may not be completely validated, and that some results don't have a solid clinical interpretation? If patients don't fully understand the information they receive, might they try to make use of it in ways that are incorrect and potentially dangerous? How do clinicians deal with patients bringing these results to them without appropriate interpretation or oversight? How do we prevent the public confusion that might come from patients armed with raw genetic results trying to make sense of findings that don't yet have a place in clinical practice? The questions, and the implications, are endless…

The train has left the station – now what?

How do we, as laboratory professionals, kick-start a conversation about these issues? First, we have to acknowledge that the train has already left the station. There's no way to recall the power provided by NGS platforms, nor should we want to do so. The benefit of generating deep and accurate data about patient samples surely outweighs our concerns about the unintended consequences that may flow from such testing. But with that said, each institution should initiate its testing with broad discussions that include the whole team – clinical geneticists, genetic counselors, oncologists, surgeons, pathologists, radiologists, and patient advocates.

> "There's no way to recall the power provided by NGS platforms, nor should we want to do so."

What should this kind of discussion entail?

- In cases where incidental findings are possible, be clear about that and agree on how patients and clinicians wish to be informed. Have a written policy to outline clearly what will be done in these cases.
- Variants of unknown significance will remain a challenge for years to come. Many national and international efforts have already produced excellent approaches to systematic handling of VUS interpretations (5–8), so follow one or more of these guidelines rigorously to ensure that all VUS from your testing facility undergo the same careful scrutiny, and that the evidence for interpretation is well-documented and stored for future reference.
- Consider sharing your VUS interpretation and data through any number of initiatives that support de-identified data sharing to improve our understanding of VUS in particular genes.
- Consider a policy for periodic VUS review – although that brings with it a host of additional questions, including the wisdom of "lookback" interpretation and how best to provide a patient with a new interpretation after initial testing.

I have some advice for patients and their families, too: have patience. Understand that those of us working in clinical labs, uncovering genetic secrets in your cells or your tumors, are doing so because of a genuine wish to help you receive more effective and rapid treatment. We are still working out how to handle these complex situations and how best to balance the never-ending struggle between the amount of information we can generate and the amount of money we can spend. We are also trying to find the best ways to decide when a piece of genetic information is really useful to a patient and when it falls into the category of "research" (interesting, but perhaps not yet ready for clinical prime-time). Appreciate that the landscape is changing rapidly, and that it will continue to do so – and that, just like you, we're doing our best to learn all we can and turn it into the best treatment and care for you.

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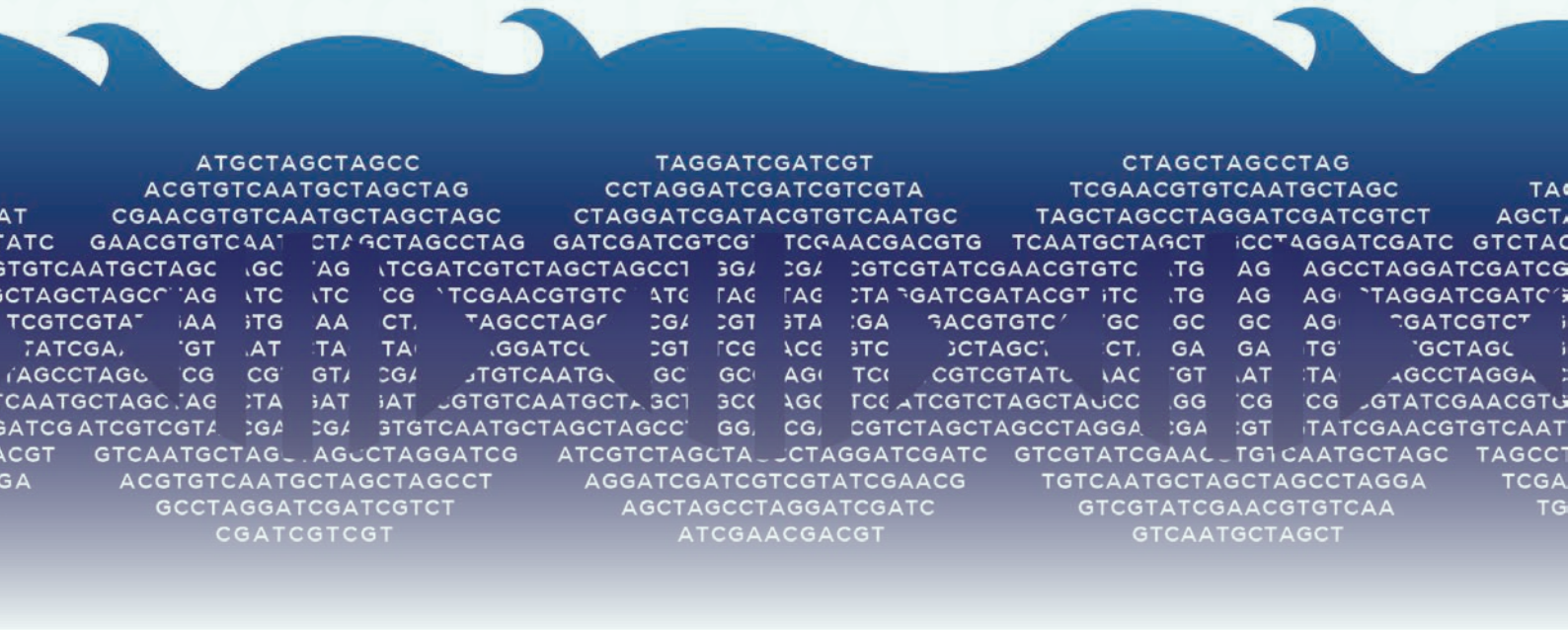

Is More Always Better?

By Jessica Mozersky

The goals of expanded gene panel testing seem beneficent – to enable better diagnosis and treatment of complex diseases with the ultimate aim of improving health. In reality, though, there remains a disjuncture between these hopeful goals and what we can achieve in practice. Our sequencing capacity continues to outpace our ability to interpret the volume of genomic data we generate. Similarly, our diagnostic capability continues to exceed the availability of treatments. One consequence is that panels may generate results with unclear significance, limited to no clinical utility, or no relation to the original purpose of testing. Variants of uncertain significance (VUS), or those associated with low- to moderate-susceptibility genes, can leave patients confused or with information on which they cannot act. Why, one might ask, does the scope and scale of gene panels continue to grow despite a limited ability to interpret them?

In the United States, commercialization and drives to increase market share are one contributing factor. The implicit ethos seems to be that the inclusion of more in a panel increases its value and market appeal – a "bigger is better" mentality. In a competitive commercial environment where companies market directly to clinicians and patients, more comprehensive gene panels may be an attempt to increase sales, rather than to improve clinical or patient utility. That's not to say that there are no benefits to panel testing, or that commercialization is the sole driver of their expansion. My aim is to remind readers that commercialization is an important factor in the increasing use and availability of panel testing in the United States – and that it may, at times, override clinical consensus and the evidence base.

There are ethical consequences related to patient beneficence and the duty to do no harm, as well as to the appropriate and just allocation of limited clinical resources. Results like VUS can cause confusion, misunderstanding and anxiety for patients – and may even result in harm through unnecessary treatment. But it is equally important to acknowledge that excessive testing can also create burdens for clinical staff and drain precious resources, for instance when large panels require additional time for interpretation and to communicate results to patients in a meaningful way. As more variants are added to panels – especially those with low risk or unclear clinical significance – there will be a parallel rise in strain on already limited resources.

Gene panels have the potential to improve clinical care – and, in some situations, they already do. At the same time, it is important not to lose sight of the complex on-the-ground difficulties they may create for patients and clinicians alike, nor of factors like commercialization that contribute to their increasing availability and uptake. This should act as a reminder: when it comes to gene panels, more is not always better.

Jessica Mozersky is an Assistant Professor of Medicine at Washington University School of Medicine in St. Louis, USA. Her research explores the ethical and social implications of new biomedical and genomic technologies including cancer genetic testing, prenatal genetic screening, whole genome sequencing, and PET neuroimaging.

Points of View

An interview with Marilyn Bui

What are the main pros and cons of genetic panel testing?

The key benefit is that it allows us to test multiple genes simultaneously, thus cutting down the consumption of tissue or sample, and reducing associated costs of running multiple tests and the time needed to return results. It's also important for guiding treatment decisions. For example, *EWSR1* is a promiscuous gene with numerous partners. Different *EWSR1* and partner combinations give rise to different types of tumors, so finding the translocation partner helps us to specify the diagnosis and to guide the most effective treatment. This is a clear example of how NGS panel testing can really make a difference to patient outcomes.

In spite of the above, there are some testing panels that are too large and include many genes which have no immediate diagnostic, prognostic and predictive value. And I feel that overall, the process of NGS panel testing still takes too long and is quite costly.

What are the main ethical issues surrounding NGS testing?

The main concern is what we should be doing with all of the data generated. There will be times when we won't find what we are looking for; for example, we may be searching for a targetable and actionable marker to treat a cancer, but we don't find it and instead we accidentally discover that the patient has certain genes that predispose them to Alzheimer's or cardiovascular disease. Should we disclose that to the patient? And do we share the raw data with them or just what we think is applicable?

Traditionally, a pathology report is generated once and represents the best understanding of the disease at that point of time. However, with NGS raw data, we may be able to interpret results later in the day, once there has been further scientific advancement. What do we do in this scenario? There is currently no guideline on how often we should go back and review these data and how we should report new findings to the patient, nor is there a requirement for us to do so. This is unchartered territory for pathologists.

How should data from panel testing be used?

This is how I think the process should work… As pathologists, we are in the best position possible to help clinicians and patients to decide if a test is appropriate and if the tissue is adequate or suitable. So, we need to be involved in ordering tests and have full access to all of the results. We should then have a central role in multidisciplinary molecular tumor board-like settings, where we discuss the results, provide full interpretation and support the formulation of an action plan. In my opinion, if any new information comes to light as a result of scientific advancement, the patient should be made aware. As physicians and scientists, we have an obligation to advance our understanding of disease. As such, patients should consent to their data being used in studies that are regulated by Institutional Review Boards; the data will, of course, be de-identified.

> "The lack of consensus guidelines that support use of the technology in practice is a problem."

What are the current unmet needs?

There is a need for further technological innovation so that the speed of panel testing is increased and the cost reduced. The lack of consensus guidelines that support use of the technology in practice is a problem too; it's important that this gap is addressed. We also need guidance on the ethics of panel testing – how we should approach testing and how we should be using the data. The bottom line is that genetic panel testing is already making positive impact on patients' care every day. It will be beneficial for us to all work together to maximizes these benefits moving forward.

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Justice Prevails?

The future of advanced sequencing isn't just molded by research into new pathways and novel diagnostics and therapeutics. For those wishing to innovate in the field, it's important to be aware of the legal battles that have been (and continue to be) fought in the courtroom around the legitimacy of certain aspects of the technique, and the precedents set by those cases. A landmark example of which is the 2013 dispute between the Association for Molecular Pathology (AMP) and Myriad Genetics (1); a legal battle that saw AMP attempt to overturn Myriad Genetics' patents for the *BRCA1* and *BRCA2* genes.

Why did AMP choose to challenge the Salt Lake City-based molecular diagnostics company? It believed that allowing genes to be patented stifled clinical research and overall biomedical progress, since the patents made it impossible for others to legally detect the genes – mutations of which increase susceptibility to breast and ovarian cancer – and gave sole supplier Myriad the opportunity to keep testing costs high. In June 2013, the Supreme Court of the United States overturned Myriad's patents and ruled that naturally-occurring DNA segments in general cannot be patented.

What this now meant was that researchers outside of Myriad could investigate and provide tests for *BRCA1* and *BRCA2* as well as other genes too; a boon for those hoping for a reduction in the price of testing or wanting to conduct new research in the field (2). This decision was such a milestone case in genetics, not only because of the implications for its future, but also the ripple effect that is bound to be felt by those holding pre-existing patents. Approximately 41 percent of existing genes are covered by nucleotide sequence patents in the US (3), so the ruling could

The Verdict Is...

A timeline of key legal cases involving genetic patents

Diamond v. Chakrabarty (1980)

When Ananda Chakrabarty's (then GE genetic engineer) attempt to patent a genetically modified bacterium was rejected in 1972, Commissioner of Patents and Trademarks, Sidney Diamond, sought the support of the US Supreme Court during Chakrabarty's challenge, which ruled that the geneticist's live, human-made microorganism was patent eligible (4). This case sparked a boom allowing institutions to patent genetically-engineered microorganisms, resulting in milestones such as the first FDA-approved genetically-engineered product – Humulin – in 1983 (5).

LabCorp v. Metabolite (2006)

In 1999, Metabolite Laboratories sued Laboratory Corporation of America (LabCorp) for infringing its diagnostic test patent. LabCorp was ordered to pay damages, but subsequently contested the patent – covering diagnostic tests of folate and cobalamin deficiencies – arguing that the technique should be covered under "laws of nature", and therefore be ineligible for a patent. The final decision by the US Supreme Court some years later allowed Metabolite to hold its patent (6). This was the first time that patenting not only diagnostics, but the methodology involved, was given a Supreme Court ruling.

jeopardize the subsequent validity of those patents if the owners decide to challenge other parties for infringing them.

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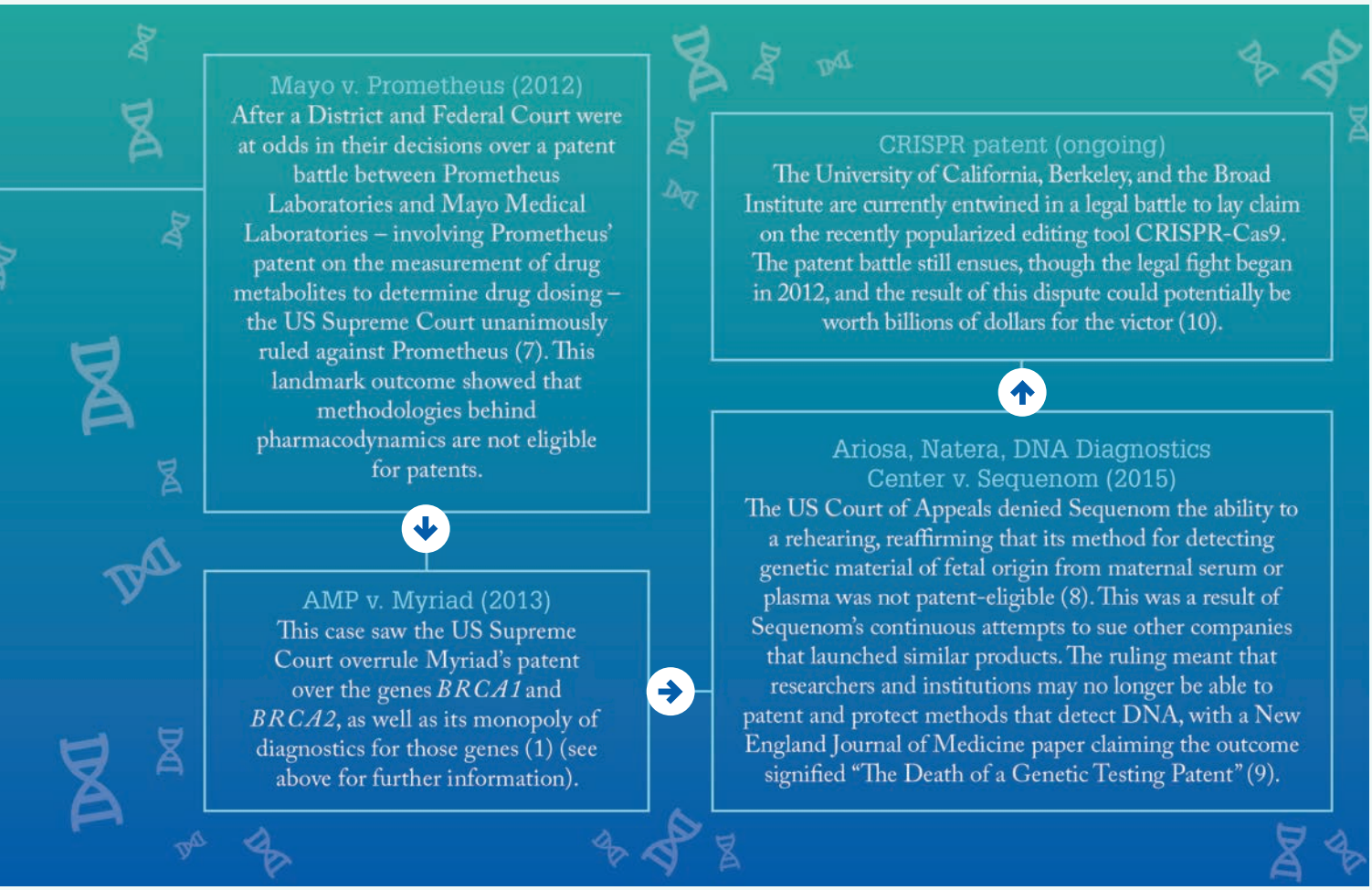

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What started out as a phone line to manage hospital admissions of dengue virus, has turned into a powerful early warning system for disease outbreaks for regions with limited internet access.

Toward Higher Standards in Viral Diagnostics

From pig pancreases to precision medicine, we rely on quality assurance and validation to keep tests accurate and reliable – but standardization also plays a crucial role

By Neil Almond

How much insulin is present in a preparation of pig pancreas for treating diabetes?

How much bacterial antitoxin is present in the serum of a horse immunized against infection?

These are just two of the questions that scientists were grappling with 100 years ago and ultimately gave rise to the National Institute for Biological Standards and Control (NIBSC) – an organization that has spent the last half-century tackling the challenge of measuring biological medicines. Although such medicines may seem crude to the modern practitioner (pig pancreas or horse serum, anyone?), the methodologies we developed then still have a lot to teach us, even in the age of synthetics and biosimilars.

In the 1980s, we recognized that the lessons we'd learnt from classical measuring challenges could also be applied to the difficulties faced by the blood industry with the emergence of blood-borne viruses like HIV and hepatitis C. Molecular amplification assays had just been invented, and we immediately recognized that they gave us the power to improve virus detection. But it wasn't as clear-cut as it sounds. When using nucleic acid amplification techniques (NAATs), we all believed that we could detect a single molecule of target sequence. But when we compared two or three results, everybody's molecule was slightly different. So the big question: was anyone correct?

NIBSC's offer to the virology community was not that we were experts in NAAT assays, but that we could make stable materials that contained a consistent amount of target sequence – standards that labs could use for comparison. One thing we wanted to target was the concept of a "copy." What is a copy? Patients, clinicians and researchers believe they know, but, in practice, each assay measures it slightly differently. We were very keen to switch to a unit that would allow us to standardize between assays.

Working with the WHO expert Committee for Biological Standardization (ECBS), we began developing reference materials that allowed the amount of target to be calculated as a relative potency in arbitrary, but defined International Units (IUs). We rolled our new standards out in the blood-borne virus industry in the 1990s and, since then, we've seen significant improvements to the quality, sensitivity and comparability of different diagnostic assays and platforms. Our next task is to extend what we've learned to a broader range of clinical viral diagnostic assays.

At a Glance

- *When it comes to diagnostics and treatments, standardization is key – but often inadequate*
- *Without the ability to compare between assays, it's difficult to determine whether differences in the results of two samples are meaningful*
- *Adequate assay standardization requires reliable units and trustworthy reference materials*
- *As technology – especially next generation sequencing – advances, we need to develop new reference materials to ensure we can keep evaluating test reliability*

Setting standards

One of clinical virology's challenges is that, because of the mobility of our patient population and the consolidation of pathology services, we have to ensure that differences between assay results are caused by actual changes in the amount of target pathogen, rather than technical differences in measurement. Disentangling those two aspects is difficult – but vital.

NIBSC has been working with the clinical virology community to develop a series of international standards. We want stable preparations of clinical targets that can last for years, and we want to be able to produce thousands of vials that all contain the same material in the same amounts. If different labs measure different contents, we need to know that the problem lies with the equipment or method, rather than the analyte itself. You may be thinking, "If each lab measures slightly differently, how can we be sure of what's in the tube in the first place?" The answer is that we actually rely on international collaborative studies – in other words, we let international expert laboratories tell us how much there is in our vials and we establish a consensus through this collaborative study process.

Making a reference material

The starting point for a new NAAT standard is a meeting called SoGAT – "Standardization of Gene Amplification Techniques." We bring together expert stakeholders from a variety of international sources and identify our main priorities. Which tests are most important for the international community? Over the last decade, for instance, it has been the herpes viruses, which affect patient health and clinical management after organ transplantation. The stakeholders tell us what they need, we bring the proposal to the ECBS for endorsement, and then we work to produce and validate a candidate reference material.

We also need to ensure that our calibration references act exactly like clinical samples – an attribute known as commutability. The volume of material required to make an international standard is too great to rely on clinical samples alone. As a result, we need to show that the standard works like a clinical sample in every type of assay in which it is used (see Commutability Conundrum). To that end, we must include a number of clinical samples as part of the collaborative study. This used to be straightforward when working only on blood-borne viruses, but now that we're dealing with whole blood, urine, cerebrospinal fluid, and so on, it's much more complex and time-consuming. Ultimately, though, once we've completed our studies and analyzed the data, we submit a report to the ECBS – and that, hopefully, results in a new international standard.

On average, the process takes about two years – but when we're dealing with an emerging virus like Ebola or Zika, we try to find ways of shortcutting the process; for instance, by reducing the number of laboratories that evaluate our standard or by developing novel evaluation or treatment approaches (1). We managed to produce an international standard for Ebola virus within nine months, which gave doctors an extra year or more of reliable clinical diagnostics.

Weaknesses to watch

Molecular diagnostic assays provide a level of specificity and sensitivity that was not previously available, and have the power to improve clinical management of patients. But that same specificity and sensitivity is also a potential weakness; subtle changes in pathogen sequence can lead to drastic differences in measurement. Suddenly, a pathogen we could readily measure might be completely missed! It's vital for diagnostic professionals to be aware of that significant Achilles heel.

An even greater concern is the "copy" concept, which we've already established doesn't always mean quite the same thing. It's not a magic number – it carries an inherent uncertainty that clinicians and patients sometimes overlook. In clinical chemistry, assay methods are very robust and the resulting numbers are reasonably accurate; the challenge with current molecular diagnostic techniques is that there is much greater potential for variability and this can be critical if guidelines state specific clinical actions should occur at a specific level.

We recently sent identical vials of

Which Words?

Standardization, quality assurance and validation aren't the same thing – but unfortunately, they're sometimes used interchangeably.

Standardization is a metrological term for the ability to compare assays performed through space and time. That might mean assays conducted in the same laboratory at different times, or assays conducted at the same time in different laboratories. The goal of standardization is to allow us to compare the outcomes of those different assays.

Quality assurance and *validation* have more to do with individual laboratories – how "good" their assays are. Those terms deal with things like reproducibility and detection. Can we repeat the same assay on the same sample and get the same result? Can the assay reliably detect its target in a wide range of clinical samples?

Commutability Conundrum

Many users see plasmids as good reference materials. The problem is that they're fine for the amplification step, but they don't look like clinical samples. We demonstrated that very clearly when we established the international standard for cytomegalovirus; we had both a plasmid and a virus that was identical in sequence, and only the virus itself covered all of the steps in the process (extraction, amplification, and so on). If your standard doesn't incorporate every step of the process, it's highly unlikely to improve the quality of the assay. It all comes down to commutability – making your reference resemble a clinical sample as closely as possible.

polyomavirus to different laboratories for quantification as part of a study. The great news is that each laboratory's results were highly reproducible – meaning that they had good quality assurance systems in place. The bad news is that there were massive (1000 fold) differences in the amount of virus each laboratory detected (2). Such disparity can significantly affect clinical management, and that's why we need to embrace the process of standardization. Quality assurance alone isn't enough; we also need reliable inter-laboratory comparison. The good news is that most of these differences disappear when they are calculated as relative potencies (2).

What comes next?

Beyond virology, molecular diagnostics are increasingly being applied to all types of infectious agents. The artificial distinctions between virologists, parasitologists and bacteriologists are being broken down at the clinical diagnostic level for infectious diseases. Instead of clinging to those distinctions, we should learn from one another by sharing new ideas, technologies and reference materials.

"It's a problem we need to solve quickly, before [NGS] gets too much bad press for 'failing to deliver on its promises.'"

Point-of-care testing is another field that's on the rise, and it carries its own set of standardization challenges. These are limited numbers of small samples that can be processed in machines designed for use in the physician's office. But they tend to be all-in-one packages; the technology is built into the machines by the manufacturers, who purportedly address quality assurance and validation – but not necessarily standardization. It's quite difficult to include appropriate reference materials when a machine may only be able to process one or two clinical samples at a time.

A final trend, which may supersede all of the current technologies being applied to diagnostic NAAT assays, is the rise of next generation sequencing (NGS). Such technology overcomes a key pitfall – namely, the fact that a minor change in a target pathogen's sequence may cause current molecular assays to miss it completely. However, it is important to note that NGS-based detection of infectious agents is no less reliant on standardization. One recent NIBSC publication showed that when we put similar amounts of 25 viruses into a tube and asked laboratories how many viruses they could detect with NGS, they each came up with very different numbers – and, not only that, the amounts they detected were also highly variable (3). In fact, some of the labs even discovered viruses we hadn't put into the vials in the first place! It just goes to show that NGS, while very powerful, is still a long way from being standardized. It's a problem we need to solve quickly, before the technology gets too much bad press for "failing to deliver on its promises."

We don't have the answers to all of these challenges yet, but at least we've begun to identify the scale of the problem. We've come a long way from pig pancreas and horse serum days – but as technology advances, we must tackle new issues as they arise to ensure that our ability to diagnose infections keeps pace.

Neil Almond is Head of the Blood and Tissue Pathogens, Adventitious Agents and Diagnostics group in the Division of Virology at NIBSC.

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Hotline to Predictive Healthcare

What started as a phone line for people worried about dengue has grown into a sophisticated early warning system for outbreaks

By William Aryitey

Epidemics are tracked in many different ways around the world. The CDC in the US uses interconnected hospital information systems to compare and integrate signals. Google Flu Trends tracks search queries for certain symptoms across time periods and locations. But in many regions high-tech methods like these aren't possible – the right infrastructure isn't in place, and it would be too difficult and expensive to implement. In Punjab, Pakistan, not all hospitals have a shared database of records, and the vast majority of the population doesn't have internet access. In these circumstances, how can we get the data we need?

At a Glance

- *In 2011, there was an outbreak of dengue fever in Punjab that escalated into the region's largest ever epidemic*
- *Unprepared for the scale, the government asked the Punjab IT Board (PITB) to launch a telephone hotline to assess patients' symptoms and prevent hospital overcrowding*
- *By analyzing data from the phoneline, it became clear that it could predict outbreaks 2–3 weeks before hospitals could confirm them*
- *A new ecosystem around the hotline has now been built to track and predict outbreaks; and the developers believe there is potential for similar systems in regions that lack widespread internet access*

Phoning it in

In 2011, there was an outbreak of dengue fever in Punjab that quickly escalated into the largest epidemic the region had ever seen. Dengue causes flu-like symptoms, rash and fever, and its severe form can be fatal, particularly for young children. "There was a sense of public panic, and authorities were unprepared for the scale of the outbreak, leading to massive queues at hospitals," says Lakshminarayanan Subramanian, a Professor in the computer science department at NYU.

> *"On analyzing the data, there was a clear trend between calling patterns and later outbreaks of dengue."*

To calm the chaos, the government asked the pioneering Punjab IT Board (PITB) to launch a telephone hotline. The hotline was initially built for people to get a quick assessment of their symptoms, determine whether they needed to see a doctor urgently, and prevent hospitals from becoming overloaded. However, it also allowed the PITB to collect data about the number of suspected dengue cases in different areas, as well as keeping track of confirmed dengue cases from the referred hospitals.

Subramanian is a longstanding friend and collaborator of PITB Chairman Umar Saif, and the two decided to launch a research collaboration to explore the hotline's potential for surveillance and forecasting. On analyzing the data, there was a clear trend between calling patterns and later outbreaks of dengue, with the hotline able to predict outbreaks two or three weeks before local hospitals could confirm (1).

The dengue forecast

"The call volume was substantial enough to create good forecasts – but the hotline itself was only the beginning," says Subramanian. The team has now built up a whole ecosystem around the hotline, to track and predict dengue outbreaks. The system comprises more than 25 departments spanning 36 districts of Punjab, a plethora of standard containment practices, dashboards to share information with hospitals, and even public health teams who are sent out to perform containment activities, such as mosquito control.

"Tracking unconfirmed and confirmed cases of dengue by locality gave us very strong signals," explains Subramanian. "Naturally, no single data source can be 100 percent relied on, and the hotline data can be skewed by people calling in about other diseases, reluctance to seek treatment after referral, and hundreds of other factors. To limit noise in the data we collect, we cross-reference it with data from other sources, such as hospital records."

The hotline now also serves functions beyond its initial remit of advice on symptoms and hospital referrals. People can report environmental conditions that encourage mosquitoes (such as stagnant water), request mosquito fumigation of their neighborhood, and make complaints. "This adds value to the hotline and incentivizes its use," says Subramanian.

Subramanian affirms that while the ecosystem they developed started as an

About PITB

The Punjab Information Technology Board (PITB) was created in 1999 by the government of Punjab to help the region harness rapid advances in the field of IT, and build an internationally competitive IT industry. Today, PITB is working on over 60 projects and services ranging from utility billing, to e-stamping, to creating livestock databases. Since 2011, the Board has been led by computer scientist and entrepreneur Umar Saif, who is widely credited as a driving force behind the Pakistani government's use of technology.

Punjab's Dengue Activity Tracking System

As well as telephone and hospital reporting, the scheme makes use of smartphones to collect real-time data on cases, mosquito breeding grounds and prevention efforts. It involves:

- More than 25 departments across 36 districts of Punjab
- 1,900 GPS-enabled smartphones used to log dengue cases and mosquito breeding areas
- 39,688 hotspots in four major districts (Lahore, Rawalpindi, Sheikhupura and Faisalabad) under weekly surveillance
- 145 hospitals with dengue data entry systems
- Over 6 million anti-dengue surveillance activities via android mobiles since launch

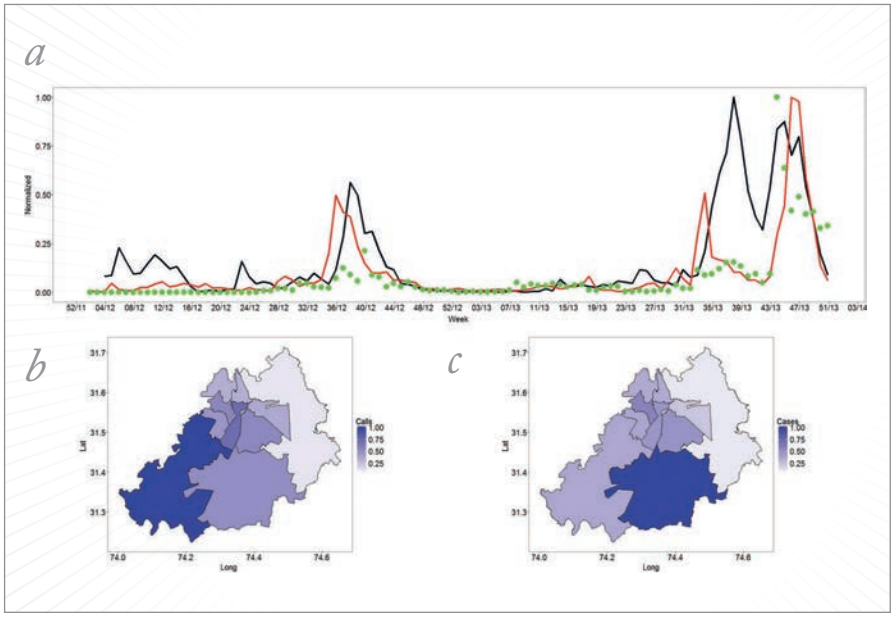

Figure 1. Trends in call volume and suspected dengue cases measured during 2012 and 2013. (a) Time series of calls (red), suspected dengue cases (black), and awareness campaigns (green points). Scale normalized by dividing by individual maximum values. (b) Density map of calls across towns in Lahore. (c) Density map of cases across towns in Lahore. The legend is normalized by the maximum value. Lat, latitude; long, longitude. Reproduced from (1).

afterthought to the original hotline, a lot of follow-up work has gone into subsequent planning and validation, involving multiple collaborations with colleagues at NYU and in the UK. "Working with leading epidemiologists, we carried out detailed studies to maximize our effectiveness and ensure we are moving in the right direction," he says.

The result is an early-warning system that is truly useful, despite its simplicity and low cost. "I think we have found a particular sweet spot of systems coming together to make our forecasting model work," says Subramanian.

Spreading the word

Subramanian believes there are opportunities for similar hotline systems in other regions that lack widespread internet access or linked hospital networks to take advantage of other forecasting tools; for example, Zika in Brazil, Ebola in West Africa, and even swine flu in certain areas.

The initial outlay is relatively modest, with most of the funds going on setting up and staffing the hotline and running public information campaigns. "The crucial first step is reaching a minimum call volume, because if you receive too few calls you won't be able to build an accurate model," says Subramanian. "Once you have the call volume, you need to get a good geographical spread and regularity of calls. If you can achieve sufficient scale and spread, you can begin to build on that initial momentum and create an effective forecasting tool."

The strategy could also be applied in developed countries, where Subramanian envisages the hotline taking the form of an internet-based hub that people can interact with – perhaps even with direct access to health professionals. There are many untapped opportunities within existing systems, too, says Subramanian. "Take the UK for example. The NHS already provides data with scale, spread, and regularity. Alone, this data might be too noisy to be

useful, but if you start putting it together with health data that other organizations collect, you could have a very powerful predictive tool. And there are so many more examples like this around the world."

"If you start putting it together with health data that other organizations collect, you could have a very powerful predictive tool."

Subramanian is convinced that there are massive opportunities to improve personal and societal healthcare using these data sources. "We just need to implement and utilize tools more effectively to improve disease prevention at very little cost."

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More Ways to Predict an Outbreak

From Mice to Men

New research shows that analyzing environmental change can help predict the risk of a zoonotic outbreak – when a disease leaps from animal to human. One such infection is Lassa fever, an acute viral hemorrhagic disease that usually infects West African rodents, but can spillover into human populations. A team at University College London looked at hundreds of past outbreaks of Lassa fever – taking into account location, land use, crop yields, weather conditions, and human population growth. The data from previous epidemics were combined with forecasts of climate change and population density to predict future prevalence (3). The team hopes the model can be applied to other zoonotic diseases to help communities prepare for the future.

Mobiles and Mobility

Researchers at Harvard University are using mobile phone data to predict the spread of dengue (4). By looking at (anonymized) call records, the investigators were able to track the movement of people in Pakistan during a major dengue outbreak. Using the information gleaned from call records, alongside climate data, the researchers developed a novel transmission model, which can accurately forecast where and when the disease will strike next. This was the largest set of cell phone records ever analyzed to estimate mobility, spanning over 40 million users.

Catching Up to Ebola

The international community was slow to react to West Africa's devastating 2014/15 Ebola epidemic. To help authorities act faster in future, epidemiologists at Columbia University conducted an analysis of data from the Sierra Leone Ministry of Health in the aftermath of the outbreak (5). They used a novel statistical model to give a detailed picture of the spread of Ebola through the country, and believe that real-time use of the model during future outbreaks could identify opportunities to curb transmission. In the midst of an outbreak, contact tracing can be too slow and cumbersome – the new model provides a faster way to track the spread of the disease, with minimal data required.

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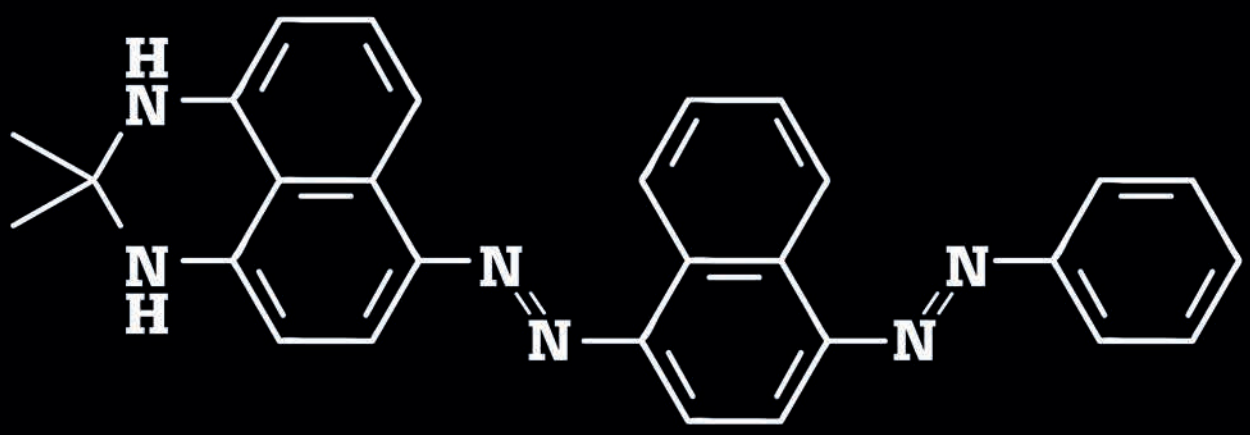

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The Secrets of Senescence
"Senescence." A word that sounds so harmless and yet, a cell in this quiet state could turn from a disease barrier into a promoter. Detecting senescent cells has been challenging, but Vassilis Gorgoulis tells us that he and his team might have just found a solution…

The Secrets of Senescence

On the back of an old technique – the histochemical detection of lipofuscin by Sudan Black B – we've built a new method for spotting cellular senescence

By Vassilis Gorgoulis

Stemming from the Latin senex, meaning "to grow old," cellular senescence is a key stress response mechanism that preserves cellular homeostasis – which makes it important in normal physiology, embryonic development, and many pathological processes.

Let's imagine a cell in a stressful environment, being subjected to various insults. The cell has various ways of responding: it can die; it can enter arrest; or it can enter a state of senescence. In the latter state, the cell remains metabolically active, but doesn't proliferate. That's why we typically consider it an anti-tumor barrier – how can a cell be cancerous if it is incapable of replicating?

But there is also a dark side. A cell that remains in a state of senescence but isn't cleared from the organism eventually presents what is called the "senescence-associated secretory phenotype (SASP)." It releases cytokines that change the extracellular environment – and can transform the cell from a disease barrier into a disease promoter. How? Changes in expression of secreted factors can cause shedding of normally membrane-bound receptors, cleavage of signaling molecules, and even degradation of the extracellular matrix (1, 2). As a result, it's vital that we are able to detect senescent cells in clinical samples.

"We're already seeing the new marker's inclusion in clinical trials, even though we only published less than a month ago."

An enzymatic answer

Until now, the scientific community has only had one way of detecting senescent cells: the senescence-associated β-galactosidase assay, which measures the activity of the lysosomal enzyme β-galactosidase. Unfortunately, the method has more drawbacks than benefits. It can only be used in fresh tissue (not archival material), and its false-positive and false-negative rates are high. Knowing that we needed a better way to spot senescence, we turned to pathology's long history for an answer.

Lipofuscin (derived from the Greek word lipo, meaning fat, and the Latin fuscus, dark) is a byproduct of lysosomal digestion. A young Danish histologist, Adolf Hannover, first detected it in 1843 in the cytoplasm of nerve cells. Pathologists have been detecting these yellowish-brown granules ever since, but it never occurred to anyone that they could serve as indicators of a stressful condition like senescence. But here's the crux: when a cell is under stress, its bioenergetics can't keep up with demand, so lipofuscin begins to accumulate. We can then detect it using a traditional histochemical stain, Sudan Black B (3) – something that modern pathologists, who rely heavily on immunohistochemistry, may have overlooked.

Like senescence-associated β-galactosidase (SA-β-gal) itself, Sudan Black B has its pros and cons. Its key advantage is that it directly detects the cell's aging process via a waste product, rather than relying on enzyme levels. It also improves upon current false-positive and false-negative rates, allows multiple simultaneous stainings, and can identify senescence not only in cell cultures and frozen material, but also in archival material – a major step forward from the β-galactosidase assay. It's a technically challenging protocol, though; you need experienced pathologists to spot the Sudan Black B-stained lipofuscin granules, especially in the presence of background "dirt." However, we believed we could remove that hurdle entirely by synthesizing our own highly pure Sudan Black B. We performed high-performance liquid chromatography on the commercially available dye, analyzed the spectrum of constituents and isolated the main component. Then we de novo synthesized its chemical analog and added biotin to it, generating GL13 –

At a Glance

- *Cellular senescence can tell us a lot about tumor behavior but, until now, we've had no good way of detecting it*
- *Lipofuscin, a byproduct of lysosomal degradation, can identify senescence when detected by Sudan Black B staining*
- *Our new method capitalizes on this, but uses a much purer analog form of Sudan Black B than has been commercially available so far*
- *In the future, we hope to roll the new compound out to pathology labs worldwide – and expand to body fluid analysis as well as tissue staining*

a new compound we can finally use in a sensitive and specific hybrid histo/immunochemical method.

Probing senescence mysteries

We are very proud of our discovery and its advantages over existing methods. I believe the scientific community will embrace it as the key method for the detection of senescent cells, especially as it can be expanded to other applications – immunofluorescence or flow cytometry, for instance. I'm also pleased that we've been able to provide something the field has needed for over 20 years: a tool for examining senescence in vivo. It's true that there have been biomarkers in the past, but none were specific; for instance, the tumor suppressor p16 has been used for senescence detection (4), but it also detects cell cycle arrest, so a p16-positive cell is not necessarily a senescent cell.

Essentially, we've added a third powerful tool to the evaluation of tumor kinetics; before, we could assess proliferation via Ki-67 and apoptosis via the apoptotic index, but now we have access to a third metric: senescence via lipofuscin. And if we see a tumor or other pathological entity with a high proportion of senescent cells, it means we have the opportunity to examine it further. Is it the "bright side" of senescence – the side that stalls tumor growth? Or is it the dark side that encourages the disease to progress? The answer to these questions lies in double stainings to detect SASP factors. If the staining is positive for SASP mediators, then tumor-promoting senescence features prevail.

When can we get hold of it?

As lipofuscin and Sudan Black B teach us more about senescence, I expect that many of the old questions will be answered and a lot of new ones will emerge. We're already seeing the new marker's inclusion in clinical trials, even though we only published less than a month ago (5). It seems the scientific community shares our enthusiasm! I've even had reviewers contact me to find out when our compound will be commercially available...

In answer to that question, I hope that the new method – and specifically, our highly pure GL13 – will become available to pathology departments in the next few months, because it's very important for the clinic. Until now, the only measure of a cancer patient's response to chemotherapy was the shrinkage of the tumor, which is caused by apoptosis. But what about the cancer cells that don't undergo that process, and instead enter senescence? The process makes them harmless but doesn't shrink the tumor. If we can include that parameter in our tumor kinetics, we can avoid giving patients chemotherapy they don't need. Better yet, we can now evaluate the effectiveness of novel therapeutic interventions that activate senescence and stall tumor growth. This is all-important: you can't kill something if you can't see it – a physician cannot choose an appropriate treatment unless he knows how the patient's disease behaves. Being able to measure senescent cells in tumors provides such an example by estimating how effective novel senescence-inducing therapies are. Moreover, this new method allows us to monitor the elimination of senescent cells in emerging rejuvenating therapies with senolytic drugs (6).

The future of senescence detection

Right now, we are on the verge of another major leap forward. So far, we've seen very positive results when testing the GL 13-mediated technique on samples of body fluids (for example, saliva and plasma), which is great because it will really boost the clinical applications. We can even combine the in situ tissue analysis with body fluid analysis for a more complete picture. And although this aspect is not yet fully developed, we believe that we will have it in the final stages as early as March!

As a final side note, I think the reason my colleagues and I were able to develop this new method is because we are also hybrids. I am a molecular pathologist – so I consider myself both a pathologist and a molecular biologist. There aren't that many of us in the world, but I think our ability to dive into both the basic and the clinical sides of research problems gives us added insight and helps lead us to advances like our new senescence test – and who knows how many others in the future?

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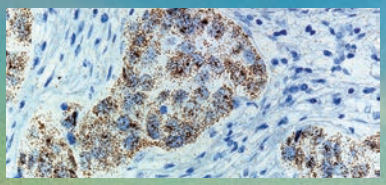

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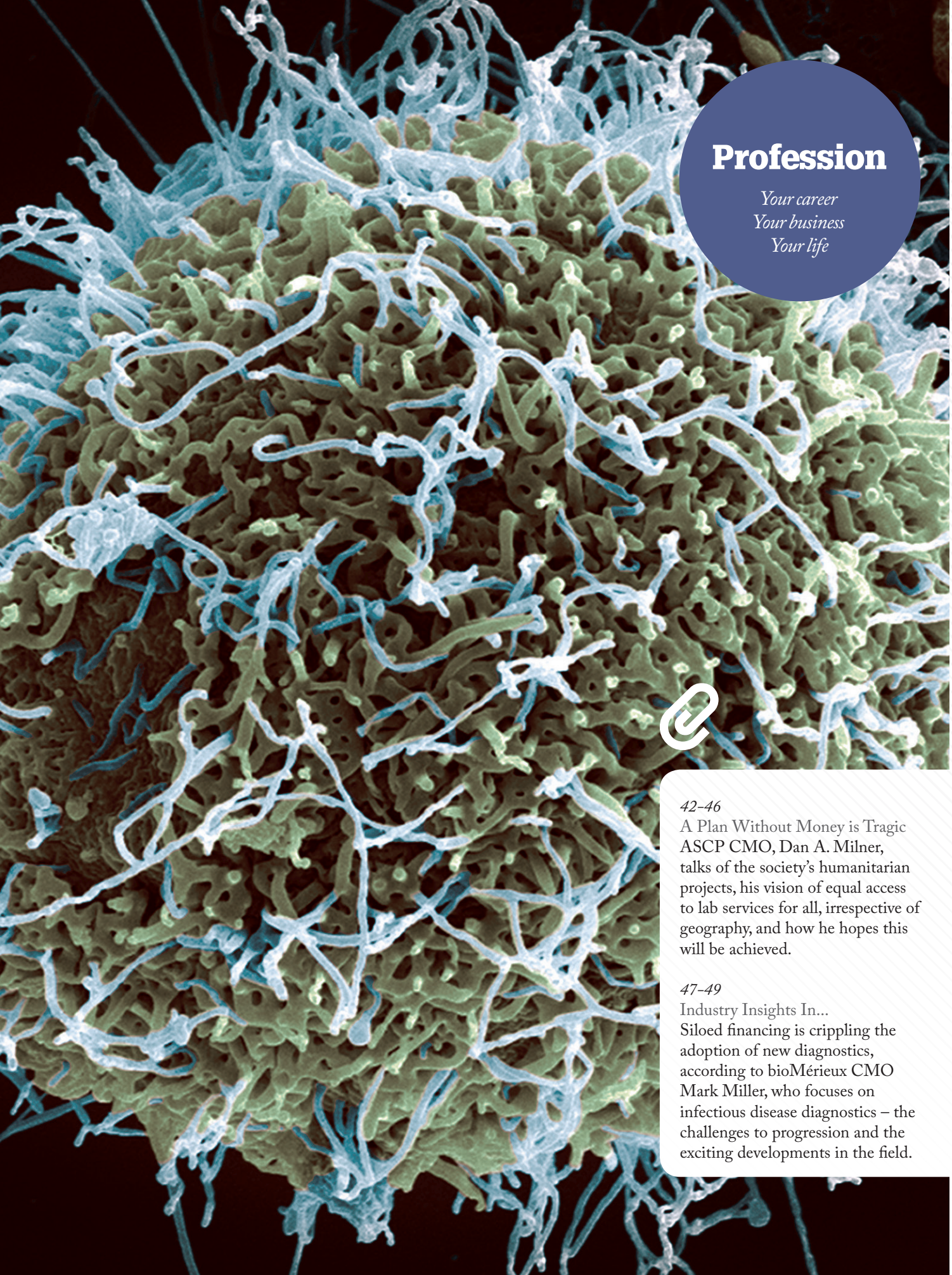

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A Plan Without Money is Tragic
ASCP CMO, Dan A. Milner, talks of the society's humanitarian projects, his vision of equal access to lab services for all, irrespective of geography, and how he hopes this will be achieved.

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Industry Insights In...
Siloed financing is crippling the adoption of new diagnostics, according to bioMérieux CMO Mark Miller, who focuses on infectious disease diagnostics – the challenges to progression and the exciting developments in the field.

A Plan Without Money is Tragic…

…money without a plan is useless

Fedra Pavlou interviews Dan A. Milner, Jr.

"This is a herculean endeavor," declared CEO of the American Society for Clinical Pathology (ASCP) E. Blair Holladay on the launch of a telepathology laboratory in Rwanda. Why "herculean"? It was made possible by Partners for Cancer Diagnosis and Treatment in Africa (a coalition announced in 2015 by the US White House and also a Clinton Global Initiative Commitment to Action), led by ASCP and supported by the National Cancer Institute (NCI) and various industry partners. The collaboration of so many stakeholders in itself is a task not to be undertaken by the faint-hearted, but the outcome is a momentous one for a country deemed severely resource-limited: a laboratory with some of the world's most advanced diagnostic systems, access to thousands of ASCP pathologist members through cloud-based systems, and a capacity of around 1,500 biopsies per day, versus 150 in a whole month previously. It's understandable why Head of the Rwanda Biomedical Center/National Reference Laboratory, Jean Baptiste Mazarati, believes that "ASCP is giving the gift of life," to his country.

At a Glance

- *Patients in resource-limited countries have no access to even the most basic laboratory services – and improving diagnostic services requires an international effort*
- *The recent launch of a telepathology lab in Rwanda – boosting capacity from 150 biopsies per month to ~1,500 per day – is an example of a humanitarian initiative led by ASCP*
- *Equipment needs for these countries must be balanced by what is most efficient, not just by what is cost-efficient*
- *Dan A. Milner, Jr., ASCP CMO, tells us what it takes to get a humanitarian project off the ground, how to make it sustainable, and why he's so focused on ensuring equal access to lab services for all*

> *"I felt helpless because I could see the problem: people have cancer, and need a diagnosis and treatment. But my hands were tied."*

This particular humanitarian initiative is just one of many that the American society is currently involved, but it demonstrates a clear commitment to improve cancer services in countries with incredibly poor diagnostic capabilities. In fact, ASCP has set itself quite an overall mission: to provide patients in underserved areas of Sub-Saharan Africa and Haiti (Botswana, Rwanda, Uganda, Haiti, Swaziland, Lesotho, and Liberia) with access to rapid cancer diagnostics and appropriate treatment – and within the next three years.

We spoke with ASCP Chief Medical Officer Dan Milner – recently appointed to lead the Partners for Cancer Diagnosis and Treatment in Africa coalition – to find out how he got involved in the gargantuan undertaking, what it takes to ensure sustainability, and what challenges he expects along the way.

When did you first become involved in humanitarian projects?

I began working in Africa in 1997 as a medical student in a rural village in The Gambia alongside an OB/GYN who ran a medical clinic out of his home. My most powerful memory from this experience was the feeling of helplessness because of the lack of diagnostics of any kind for every patient we saw. I greatly enjoyed this first glimpse of Africa, but was determined to return when I could actually make an impact.

My work in Malawi was particularly important to me. From 2000, I worked with Terrie Taylor on an autopsy study of cerebral malaria and continue to work with her for analysis of our robust data set. My exposure to the disease in its most mortal form reinforced my desire to always look for impact in my activities in global health. It was during these visits that I began working with the pathologists at the University of Malawi College of Medicine to review surgical pathology cases. From day one at the scope, almost every case I reviewed was cancer. Some years later, we pulled data from 1997 to 2007 for all surgical pathology and showed that 75 percent of the diagnoses were non-infectious – and most of those were cancer. Again, I felt helpless because I could see the problem: people have cancer, and need a diagnosis and treatment. But my hands were tied, and I could not do anything other than report what I was seeing.

How can we successfully provide service support to resource-limited countries?

There are three key elements that are very simple, but essential for success:

Top left: Jean Baptiste Marazarati (fourth from left) and E. Blair Holladay (second from right) cut the ribbon at the opening of the new laboratory in Rwanda last fall. Dan A. Milner is second from left. Top right: E. Blair Holladay views patient cases with Deo Rubangaza generated with the new equipment. Bottom: The infusion center, a collaboration of the Rwanda Ministry of Health, Partners in Health, and Dana-Farber Cancer Institute, treats all patients diagnosed by the ASCP project.

i) Partnerships. You can't do it alone and you must have buy-in from a country's partners, including the Ministry of Health (MOH), the pathologists, the hospitals or health centers, and the financial backers for the effort. Each country is unique in terms of who these partners are, which leads to...

ii) Assessment. You can't make an implementation plan without knowing what's going on in the country. Who is there? What do they have? What do they need? These are questions that have to be answered by the country partners with guidance from external experts so that a culturally and politically acceptable solution can be created. And that needs…

iii) Funding. Whether through donations of equipment or money, there must be fiscal support for a project because pathology is expensive – but not so expensive that we cannot justify the cause. In fact, a fully functioning

E. Blair Holladay, Dan A. Milner, Butaro staff, and partners toured the cancer treatment wards at the Butaro District Hospital.

anatomic pathology laboratory is one of the most valuable investments a hospital can make because of the value of tissue diagnostics across so many aspects of medicine. And it has immediate impact. But these are real dollar costs and physical or chemical processes that don't have "work-arounds."

Can you tell us about your success story in Rwanda?

As a faculty member at the Brigham and Women's Hospital (BWH), I served as the liaison for Partners in Health (PIH) cases – biopsies from patients in PIH sites around the world that came to the Brigham for pro bono processing and review. We began with a trickle – a few cases a month at the most would be sent, which began to expand linearly and then exponentially as we became the primary diagnostic force for Rwanda and Haiti. By 2011, it was clear to the whole team (PIH/Dana-Farber Cancer Centers/BWH) that something had to be done for both Haiti and Rwanda from within. Cholera had just hit Haiti, and one of my mentors and funders, Larry Shulman from the Dana-Farber Cancer Institute (DFCI), sent myself and a colleague, Jim Pepoon (a pathology technical expert), to Rwanda to assess the situation. Six months later, a fully functioning anatomic pathology lab was opened. And now, it also has immunohistochemistry and telepathology, allowing us to bring a four- to six-month turnaround time down to five days. What I learned most from this experience was the following (a phrase that I recite frequently): "Money without an implementation plan is useless; an implementation plan without money is tragic." We can turn the useless into the useful by rectifying this tragedy and acting for impact.

How do you ensure project sustainability?

The Partners for Cancer Diagnosis

and Treatment in Africa initiative includes a medical education steering committee, as well as partners whose main mission is education. As noted earlier, an assessment of a country's current and future needs for staffing is part of the process. Plus, we work with in-country schools and partners to ensure that there is a plan in motion to create the sustained workforce needed to do the work going forward. In a similar process (once again working closely with funders and in-country partners), ASCP creates sustainable budgets to support pathology work by making the business case for pathology, illuminating potential public-private partnerships, and working directly with the MOH of a given country to prioritize and value pathology services.

Let's look at the sustainable delivery of telepathology services as an example. The initial stage of the project includes funding and delivery of equipment from our technical partners in parallel with the support of a team of up to 15 ASCP pathologists per country, who provide diagnostic support. The equipment itself has a finite life (~10 years or less), so we envision our involvement with a given country to be ~10 years. During that time, we monitor the plan and provide opportunities for training and education for the workforce. In addition, each country must have a national cancer plan, which should include pathology services across multiple sites. Wherever possible, ASCP will assist with the implementation through training, partner connections, and field-based support. The end goal is for there to be enough centers of excellence in cancer care to support 100 percent of a country's population.

What are the main challenges when setting up a program in a resource-limited country?

We do encounter some locked doors. The key to these doors may be approval from a governmental agency, funding, personnel training, weather conditions, disease control, and so on. Sometimes these are predictable, so we do our best to get the keys in advance; for example, we work directly with the MOH when possible. Others cannot be foreseen (for example, the cholera outbreak in Haiti) and require patience and interim solutions until we can get back on track with our implementation plan. But, going back to my essential elements, if you have partners, have done an assessment, and have the funding, everyone actually wants the project to move forward and shares a common interest in finding the keys as quickly as possible. Without partners, assessments, and funding, the keys are likely lost to you forever and the project stops.

Is support from industry sufficient?

We need more support from many partners to help with all aspects of capacity, including equipment, personnel, and infrastructure – so industry is not alone. But we do need everything for standard pathology, including grossing hoods, tissue processors, embedding stations, microtomes, slide stainers and coverslippers. We need microscopes. We need storage equipment for blocks and slides. We need computers and software to manage the laboratory. We need reporting systems to get the diagnoses back to the patients and care givers.

It's important to recognize that equipment needs must be balanced by what is most efficient – not just by what is cost-efficient. As an example, if you walked into a microbiology laboratory today, you would see lots of hoods, incubators, storage for biochemical tests, antibiotic discs, microscopes, and so on – the bulk of which would be for the identification of bacteria. You might also see a mass spec and/or an automated biochemical reader. If I were building a microbiology laboratory in a resource-limited setting, I would start with a mass spec and an automated biochemical reader. Those two pieces of equipment alone can provide an identification and antibiotic resistance pattern for more than 99 percent of bacterial infections, and they work on fungi, too. By leapfrogging the incremental older pieces and jumping directly to the most efficient current system, we can make massive improvements and have a great impact in resource-limited settings. Molecular tests that dictate therapy for cancers are another example. In our current iteration of the project, we are working with standard laboratories to use telepathology to provide diagnostics. But tomorrow, a tool could come along that solves cancer diagnostics for a certain group (breast or cervix, for example), and we would consider adopting that technology if its impact can be measured.

"By leapfrogging the incremental older pieces and jumping directly to the most efficient current system, we can make massive improvements."

However, no single partner can provide sufficient numbers of any one item to meet all of the needs. We also need clinicians to be trained to identify cancer; surgeons to be able to biopsy or

remove lesions; oncologists to be able to act on our diagnosis; and a cadre of ancillary health workers to support and care for our patients. Again, we have identified partners, but do not have enough to cover what we could do.

We either need to recruit more of the same kind of partners to expand what we can do, or publish what we are doing in a "how to" manner, so that others can create and execute similar approaches. After all, this not a competition nor a process to seek glory; it's about providing care for people who need it, which we have a moral obligation to do.

How easy is it to recruit pathologist volunteers?

Telepathology has made it possible for many more people to be involved in these projects than previously. And although technical expertise is also needed on the ground, pathologists can provide a great deal of support remotely. ASCP pathologists and laboratory professionals also tend to have an overwhelming enthusiasm to do what's right and to support patient care anywhere in the world. What more motivation does anyone need to get involved in an effort than to know that a project will save lives?

Your goal is for all cancer patients in Africa to have access to cancer diagnostics and treatment. How can this ambitious objective be achieved?

It requires functioning anatomic pathology laboratories with rapid turnaround time and accurate diagnostics, which ASCP plans to provide directly, with the support of its telepathology network. It also requires health systems that allow us to i) find patients, ii) screen patients, iii) biopsy patients, iv) understand diagnoses, and v) treat patients. These components are assisted by ASCP members' talents, but also require a large, ever-growing group of partners from all aspects of the cancer spectrum, including clinicians, oncologists, surgeons, radiologists, Ministers of Health, Ministers of Finance, and the people of a given region.

For each site in the regions of Africa that we serve, we have three key goals to achieve:

i) that very first slide is scanned and viewed by a pathologist in the US from a new site
ii) all slides will have a diagnosis, receiving average, within five days of collection
iii) the percentage of patients receiving treatment increases in line with the population covered by a given lab.

Our long-term goal is to show a reduction in mortality from malignancy in any given region – but that's in no way easy. At first, we will expect mortality rates from cancer to increase, simply because patients who would not have previously presented to a doctor will show up (and likely at a late disease stage). As the systems are built and communities are educated about cancer, people should start presenting at earlier stages and mortality will start to drop very quickly – but it might take 5–10 years to get there.

How did it feel to be appointed CMO of ASCP?

As I have told my CEO several times, "this is my dream job." Many people talk about leaving their mark on the world, which I find to be self-centered and not in the least humanitarian. I am, at heart, a humanitarian devoted to the elimination of poverty and equity across all human populations. I want to have an impact and know that my actions, activities, and interactions are saving people's lives. Those people may never know what I (or the thousands of other people involved in this or other projects) have done to impact their life. But they have their life to live, which is hugely satisfying. Having the ability to focus on global health with our members and to work with them on a daily basis to create this and other programs is truly remarkable. Every day, I remind myself that I cannot take this work for granted and must give it my best – how low is the probability of me being in this role and in a position to do the things that we can do?

Having grown up in a small town in Alabama, I experienced poverty at an early age, both directly and indirectly. My father's generosity, despite our own unstable financial situation, was an inspiration to me and left a very strong impression. I feel happy in my heart and comfortable in my skin when I can say, "I saw what was happening, and I did the best I could do to make it right." That may be buying a meal for a homeless person, delivering school supplies to an orphanage in Malawi, or creating a diagnostics system for patients in Africa. But in all those settings, I feel the natural need to reach out and help others as best I can within my abilities at all times.

If you could fast-forward 10 years, where should we be?

I want a mother of three in rural Angola to notice a lump in her left breast and think to herself, "Oh, no big deal. I'll go to the doctor this afternoon. He can take a piece of this lump and tell me what it is later this week." I want pathology to be an afterthought. And that's not that I want people to take it for granted, but rather I want them to understand its value and know that they have – and deserve – equal access to the care that they need.

Dan A. Milner, Jr. is Chief Medical Officer of the American Society for Clinical Pathology.

Industry Insights In…

…Infectious disease diagnostics

Fedra Pavlou interviews Mark Miller

If you scour decades' worth of health stories in consumer literature, you're likely to see a great number of articles on "groundbreaking" developments in the treatment of a given condition. Fast forward to the present day and, though these (often sensationalist) stories still abound, you are more likely than ever before to read about a new diagnostic test or screening program in your daily newspaper. Much of this attention has been driven by the molecular diagnostics revolution – particularly in oncology, but recent public health emergencies, such as Ebola and Zika, have also had an impact. And though increased awareness of the criticality of good diagnostics is great for the field, there are downsides. We spoke with Mark Miller, Chief Medical Officer of bioMérieux, to find out more...

At a Glance

- *High profile public health emergencies have raised awareness of the importance of infectious disease diagnostics, but huge unmet needs still exist, especially in low- and middle-income countries*
- *Validation of diagnostics is also a big concern; many laboratory tests have not been adequately validated*
- *It is difficult for lab professionals to decipher which tests have high-quality performance; medical education is needed to support effective decision-making*
- *There are many exciting innovations in infectious disease testing, but a change in the approach to hospital financing is necessary for the field to progress and for patient care to be improved*

Would you agree that high-profile infectious disease outbreaks have raised public awareness of diagnostics?

Absolutely. Though diagnostics have always been a major preoccupation of bioMérieux and many other companies, the general infectious disease focus for many decades has been on therapeutics and vaccines, which is great, but diagnostics have had to take a backseat. Vaccines for Dengue, Zika, and meningococcal virus, for example, need to be accompanied by the proper diagnostics, otherwise we would never know if the vaccines are successful. Moreover, we would remain ignorant to the true prevalence of these conditions without diagnostics. Finally, I believe the lay public, clinicians, and labs are all now better sensitized to the dangers of ignoring diagnostics.

What does increased awareness mean for diagnostic test development?

There's a huge buzz around infectious diseases right now, with many researchers and manufacturers wanting to ride that wave. However, operating in the diagnostics space requires thorough pre-market testing and validation to assure high-quality performance – but not all companies or labs are equipped for this task. The outcome? There are now a plethora of diagnostic tests on the market – some CE marked, others awaiting regulatory body decisions, some with very limited regulatory oversight – and it has become hard for labs to figure out which tests are reliable and which are not.

As a manufacturer operating in the infectious disease diagnostics business for more than five decades, we place a lot of emphasis on validation and ensuring that we achieve the performance that labs and patients expect. And though such stringent processes favor companies with the right level of resources, such as ours, smaller companies can struggle with the complicated regulatory landscape – that's a tragedy, because good tests can sometimes be impeded from entering the market.

Lab-developed tests (LDTs) further muddy the water. Some labs have excellent internally-validated LDTs but others do not, which raises the issue of heterogeneity of both performance and the definition of validation. Should validation be local only? And how much validation is required before a test can be used within a hospital, lab or clinic? I know that the FDA is working on harmonization of validation standards, but it's a huge quagmire...

> *"Many people are out there making claims, but very few of those claims have actually been validated."*

What are the consequences of inconsistent validation practices?

I can offer an extreme example. During the Ebola crisis, a huge number of tests were being developed to diagnose the virus, but not many of them were being validated in the field, which meant that their performance (and by default, the accuracy of the result) was questionable. This is dangerous, but just one example that can be applied to so many other targets – Zika, antibiotic resistance… Many people are out there making

claims, but very few of those claims have actually been validated.

It's a big problem and, unfortunately, companies with good tests tend to be viewed with the same level of distrust. And that's not surprising; sometimes the labs, clinicians and even the regulatory bodies (until they go through the data) are not knowledgeable enough to differentiate between them.

How can the validated tests be distinguished from those that are not?

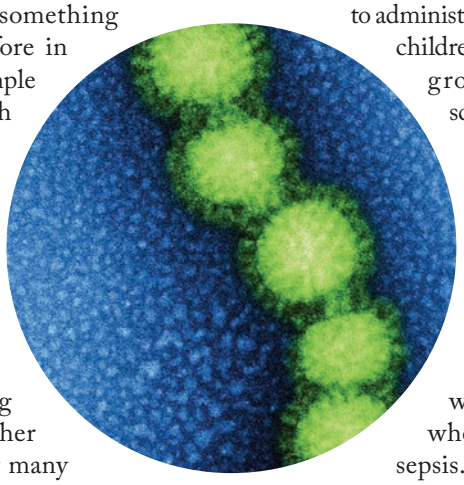
In general, it's back to the old adage of 'buyer beware.' When considering a test, laboratories must ask about performance criteria and validation – and they need to ask if the test has been validated in the population and for the disease of interest. It can be a minefield.

How can the complexity of the regulatory process be improved?

I think regulators need to apply the same approach to diagnostics as they do to therapeutics. For example, there are shortcuts and priority programs for orphan drugs, and for treatments with high medical value, such as novel antibiotics for multi-drug-resistant organisms. Let's create criteria that would make a diagnostic test important and prioritized, and incentivize its development and validation; incentives could be tax-, regulatory- or research-related. Reimbursement is also key. A diagnostic should be reimbursed based not only on the technology used but also on its medical value – whether that be a reduction in length of hospital stay, reduced antibiotic use, improved patient outcomes, and so on. This approach does not currently exist for diagnostics, but those of us working in industry are all pleading for it.

But haven't discussions around diagnostic reimbursement already begun?

They have, but they are in their infancy. People acknowledge that the system is not functioning properly, and we're starting to see a link being made between health technology assessments (HTAs) (which assess diagnostic tests) and the actual impact on a patient, hospital or society. That's not something that's happened before in the US. A good example of where the approach is already being applied is the UK's NICE. There's also a new program in France that links HTAs and health economic outcomes to reimbursement; and the same thing is happening in other parts of Europe. But many countries are still using the old model, where for instance every molecular test is reimbursed irrespective of patient value. And that's something that really concerns us, because diagnostics are not all the same and they don't all bring the same medical value.



Collecting that data and making the cost argument is going to take a long time…

Yes, it will. And it's also expensive and complicated. For instance, most HTAs conducted by NICE take between one and two years and require a tremendous amount of resources. And that means that we have to wait for a government, an agency or somebody like the WHO to take an interest in evaluating diagnostic assays. We, the diagnostic companies, can only do so much; we certainly can't conduct health outcome assessments with the breadth and scope that governments desire.

What are the areas of current unmet need in infectious disease diagnostics?

Without doubt, the biggest infectious disease killers in the world today: diarrheal and respiratory diseases. Both are killing children in Africa, South America, and Southeast Asia. We can make a huge difference by diagnosing the pathogen among the 15, 20, 25 that are possible, and within a sufficiently short timeframe to administer treatment so that children can eat properly, grow, go back to school, and survive.

Even basic infectious disease tests are still not performed in many countries in Africa; for example, blood cultures for people with a high fever who are at risk of sepsis. Why not? Well, the reasons are complex and relate to price, regulation, the healthcare system, payers, and reimbursement. The bottom line is: low- and middle-income countries don't have these basic tests, and that should be bothering everybody. I would love to see good quality, basic infectious disease diagnostics in all countries.

What are the most interesting areas of innovation in the field?

There are three key areas that I feel are really making a difference right now. The first is the so-called 'syndromic approach' to testing, which is one of the most interesting developments that this field has witnessed in the past five years or so. In infectious diseases, the classic approach involves guessing what the pathogen may be and then selecting the specific test(s) for it. But the introduction of new technology with multiplex nucleic acid detection capabilities – multiplex PCR – is allowing us to test for up to 30 different pathogens or so simultaneously, including antibiotic resistance. Not only is this allowing us to get at the root of what's causing the patient's issue, but it's reducing

the time that it takes us to get there. So we finally have diagnostic tests that mimic the way that clinicians approach patient tests – by syndrome rather than by pathogen guesswork – and I think this is very exciting.

The second relates to speed and simplicity. We now have the technology to conduct a test at the point of care. These improvements are actually allowing clinicians to come to a treatment decision while the patient is still present, instead of having to send them home and wait a period of time before the result is returned. The value of this advance cannot be underestimated: it will lead to better patient management, better diagnosis, and probably, better use of antibiotics – whether it's not using them at all or using more focused ones instead.

Finally, mass spectrometry. It's an accepted fact that the technology – and its ability to not only differentiate between pathogens but to do so in 15 minutes versus days – has revolutionized the labs where it has been introduced. And yet, there are still huge numbers of labs, in the US and elsewhere in the world, that have not adopted it. I think that's largely owing to a lack of understanding among the lab medicine community around its capabilities – and that's not their fault. After all, they are flooded with new developments almost every day.

So, awareness of new developments among the lab community is an issue?

I believe it is. Many hospitals and labs actually still use the same microbiology techniques they've been using for 100 years! Perhaps the main reason is that microbiology and infectious disease diagnostics are only seen as a small branch of lab medicine – relegated to what I call "third cousin" status below cancer diagnostics and tissue pathology.

For instance, I read the results from a survey conducted in France that showed that general physicians were still not using simple rapid tests for group A strep (1). They were vastly under-utilized even though they are cheap, perform well and have been available for years. Why? Many of them didn't know they existed, some weren't aware of the increase in performance, others were worried about reimbursement, and so on.

If you take the other extreme, we can look at highly complex tests like multi-PCR syndromic panels and novel assays like innovative biomarkers for sepsis prognosis and kidney injury evaluation. Despite the availability of these valuable tests, there is not enough known among the lab community to ensure the widespread use of these diagnostic tests for the right patient under the right conditions. I think laboratory professionals need help and we, as manufacturers, certainly have a role to play. How? By developing evidence-based medical education. If you just look at procalcitonin, which is probably the single most useful biomarker in infectious diseases today, it took over 10 years for significant market adoption. There was a lot of skepticism among clinicians and labs before they realized that there was enough data out there to trust it and use it. Hopefully, we're a little faster today at getting the message out, but I still think that more education is needed in the infectious disease space.

What impact do different financing models have on the adoption of new techniques and tests?

Siloed financing is the single biggest barrier to adoption of novel diagnostics – at least, in hospitals. It's a huge problem. Those hospitals that are truly progressive are the ones that break down the silos between departments; where administrators look at budgets transversely across a hospital and allow a department to benefit from the savings made in another, or conversely, where an investment in a particular department will directly benefit another. For example, you could demonstrate that a $50,000 machine in the pathology lab reduces the length of hospital visit in an emergency department by 12 hours, making it a worthwhile investment. There are far too many hospitals today that still have the antiquated silo financial structure, where each department is responsible for its own budget and are under continuous pressure to save money and to justify spending. This is a Neanderthal approach; it's damaging, inhibitive, and it has to go.

"Siloed financing is the single biggest barrier to adoption of novel diagnostics."

I think there are enough good models out there of how it can work successfully, and hopefully that ship will turn around eventually.

What can industry do to help?

I can't stress enough the importance of investment into R&D. Companies can really help address the issues that I've been discussing by continuing to innovate in infectious disease diagnostics, to drive down the time to results, and to increase their medical value. We cannot just content ourselves with selling products; we have to sell solutions, and investment in infectious disease diagnostics must be an ongoing focus.

Mark Miller is the Chief Medical Officer of bioMérieux.

Reference

1. *J Carlet, P Le Coz. "Let's save antibiotics together", Proposals of the Ad Hoc Working Group for the Preservation of Antibiotics. Ministry of Social Affairs, Health and Women's Rights. (2015) http://bit.ly/2k5Zvr4*

Social Superstar

Sitting Down With... Jerad Gardner, Assistant Professor of Pathology and Dermatology at the University of Arkansas for Medical Sciences College of Medicine

What attracted you to pathology?

When I was 17 years old, I was a technical assistant at a private laboratory in Florida – Pensacola Pathologists. My friend's mom was a histotech and lab manager there (Shari and Janet, I'm eternally thankful for you both!). It was serendipity. I watched gross dissection and autopsies. I coverslipped slides. I peered into the microscope and watched the pathologists instantly decide if something was cancer or not…as if by magic! I loved all of it. Frederick Nora was one of the pathologists I worked with there. He was enthusiastic and had a great sense of humor. I once asked him if he liked being a pathologist, and he said, "Jerad, I come to work, have fun, and they pay me for it!" I'm not sure if he remembers that conversation or even remembers me. But I've never forgotten that succinct but poignant quote, and I now feel exactly that way about my job as a pathologist. It was only years later, after I became a bone and soft tissue pathologist, that I made the connection that Dr. Nora was actually *the* Nora of Nora's lesion (bizarre parosteal osteochondromatous proliferation [BPOP])! Pathology truly is a small world.

You encourage others to incorporate social media into their professional development. Why?

So many wonderful mentors helped make me into the man and pathologist that I am today. Though I cannot repay that debt, I can pay it forward. Investing in professional social media use has been one of the best career decisions I've ever made. Now I want to help ensure that other pathologists make the right choice about using social media, too. If we as pathologists all use it, we will have a powerful public platform from which to educate, advocate, and promote good patient care worldwide. This is our chance. We must speak up together boldly on behalf of our field and our patients. So many people from all over the world have told me how much they appreciate social media posts by pathologists, how our posts have helped them take better care of their real-life patients. I've met sarcoma patients via Facebook support groups and it has changed my life, my research, even how I practice medicine. I want every pathologist to experience these amazing things. I truly think our world would be a little bit better if more pathologists used social media. I have more experience with it than most other pathologists in the world, so I feel it is my duty to share those experiences with the rest of the pathology community and mentor and encourage them in their social media development in any way I can. I am paying my debt to my mentors and hopefully inspiring another generation of pathologists.

"I've met sarcoma patients via Facebook support groups and it has changed my life."

Is there one achievement that you are most proud of?

I'm most proud of my work with sarcoma patient support groups on Facebook (see http://tp.txp.to/JG/patient/support/groups). The chance to educate and learn from rare cancer patients, to help empower them, to raise awareness about their diseases, and to collaborate with them in designing research focused on their own tumor types has been priceless to me personally. I believe this could revolutionize the future of rare cancer research, and it is a powerful innovation that has zero cost! Pathologists could be at the very center of this by being involved in these groups just as I have been. We now have an IRB-approved prospective research study of one of these sarcomas (dermatofibrosarcoma protuberans) that is currently ongoing; we can recruit patients and obtain long-term clinical follow-up info directly from them via Facebook. It's a new world, and it has been truly amazing to see all of this happen so quickly. My dream is to one day see every type of rare cancer have its own Facebook patient support group, where patients can find comfort and empathy from each other and from pathologists and other doctors who will collaborate with the patients in the fight against their disease.

What would you say to those who think pathology is a job for people who don't like to communicate?

Well first I would laugh out loud for a long time. And then after I composed myself and wiped the tears from my eyes, I would gently explain to that person about all of the different ways that pathologists demonstrate excellent communication skills. From how we carefully craft the wording of our pathology reports, to how we interface with our colleagues in other specialties at tumor boards or during frozen sections, to how we share our knowledge with medical colleagues using social media. I would show them my YouTube videos about how pathologists use patient support groups on Facebook to interact directly with rare cancer patients (see http://tp.txp.to/JG/angiosarcoma). I would show them my survey data of over 1,100 of my followers, the vast majority of whom say that they benefit from my social media posts about pathology (see http://tp.txp.to/JG/social/media/evaluation). And I am just one out of the thousands of pathologists on Twitter, Facebook, and Instagram who are doing similar things; bridging the knowledge gap between pathology and the rest of the world via social media. And then after all of that I would ask: "So what was that again you were saying about how pathologists don't like to communicate?" ;-) <Mic drop>

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