JUNE 2020 # 66

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Local News; Global Views

Will the COVID-19 pandemic bring us closer to global healthcare?

The months of May and June have been divisive for the world. Some states and countries are lifting lockdown restrictions; others are doubling down. Some people have access to free testing and effective contact tracing; others feel underinformed, unsafe, or have concerns about privacy and security. Whereas many administrations have laid out clear plans, others are still subject to uncertainty, criticism, or a lack of clarity in their messaging.

Amidst the whirlwind of change, I can't help but recall a message from epidemiologist Keren Landsman in last month's feature article: "Because future pandemics are an absolute certainty, I hope that we learn to work together as a global community." Landsman calls for a coordination of health efforts worldwide – "a truly global health system." Is such a thing possible?

At the moment, perhaps not. Our approaches to healthcare management are too fractured to unify easily, especially under the added pressure of a pandemic and the delayed workload waiting for us at its end. But despite the differences in logistics, one aspect is shared across the world's healthcare systems: practitioners' dedication to their patients.

On social media, I see pathologists and laboratory medicine professionals pulling together – sharing knowledge, consulting on cases, drawing from their own experiences to offer advice. In my interviews and inbox, I see a collection of people eager to help in any way they can, whether by freely providing their expertise to the public or sending surplus reagents to laboratories in need. In the news, I see doctors stepping outside the bounds of their specialties to offer support on the wards treating COVID-19 patients while scientists repurpose their work to help with everything from virus proteomics to vaccine development.

It's true that we face obstacles in converting the healthcare systems of 195 countries into a single, functioning entity – but we shouldn't let that blind us to the ways in which we already cooperate around the world.

Our health systems may not (yet) be global – but, increasingly, our patient care is.

Michael Schubert
Editor

Contents

On The Cover

A collection of tools and devices to represent the training and tissue preparation that take place in the lab..

Feature

In Practice

NextGen

Reports

Profession

Sitting Down With

the Pathologist

ISSUE 66 - JUNE 2020

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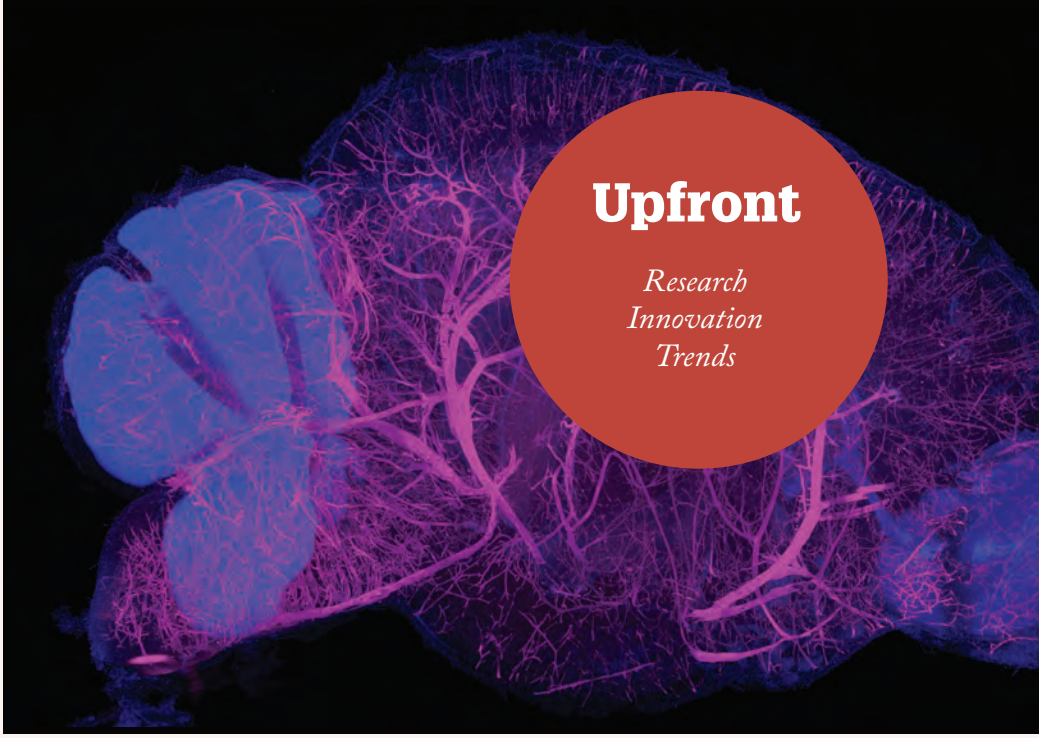

A New Dimension

The staining technique that opens the door to 3D histopathology

3D tissue imaging holds great promise – but although tissue clearing can produce stunning images, it has little scientific value on its own. To study specific tissue and cell types, a versatile staining and labeling method that works across a range of staining agents and antibodies is needed. A team from Japan have now developed exactly that, using their technique, CUBIC-HistoVIsion, to stain and image not just tissues, but even an entire mouse brain (1). Etsuo Susaki from the RIKEN Center for Biosystems Dynamics Research explains more.

How did you develop the staining method?

By carrying out physical and chemical analyses, we discovered that the physicochemical properties of biological tissue can be recreated in electrolyte gel. Toyoichi Tanaka first described biological tissue as a gel at the Massachusetts Institute of Technology in the 1980s – and we were excited to rediscover his work.

We selected an artificial gel to mimic biological tissue and experimentally evaluated various staining conditions to establish a fine-tuned 3D staining method called CUBIC-HistoVIsion. Our bottom-up design approach works with over 30 different antibodies and nuclear staining agents.

What is the new technique's significance?

Immunostaining is a powerful way to detect cell types, protein expressions, protein modifications, and protein localization in tissues. 3D imaging can reveal the precise location of these signals with the same level of detail as transcriptomics. The technique can be used to identify new cell states or cellular connections or to collect information on cell types and their positions in the body. It can also be applied to projects such as the Human Cell Atlas or the Human Protein Atlas to assist with mapping locations and relationships.

We have already used our method to compare whole-organ anatomical features among species – including imaging a mouse brain, half a marmoset brain, and a square centimeter of human brain tissue. Our previous work, in which we applied this technique to human lung and lymph node tissues to detect malignancies, has underlined the potential of 3D histopathology (2) – and further research will improve the diagnostic accuracy and objectivity of 3D clinical pathology examination in the future.

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TIMELINE

SPECIAL SERIES
Infectious Disease

The Story of a Diagnostic Test

The development of a rapid, sensitive conjugated polymer nanoparticle test for COVID-19

Weeks 1–3

Monomer selection and prototyping.

Optimizing linkage protocol.

Week 4–5

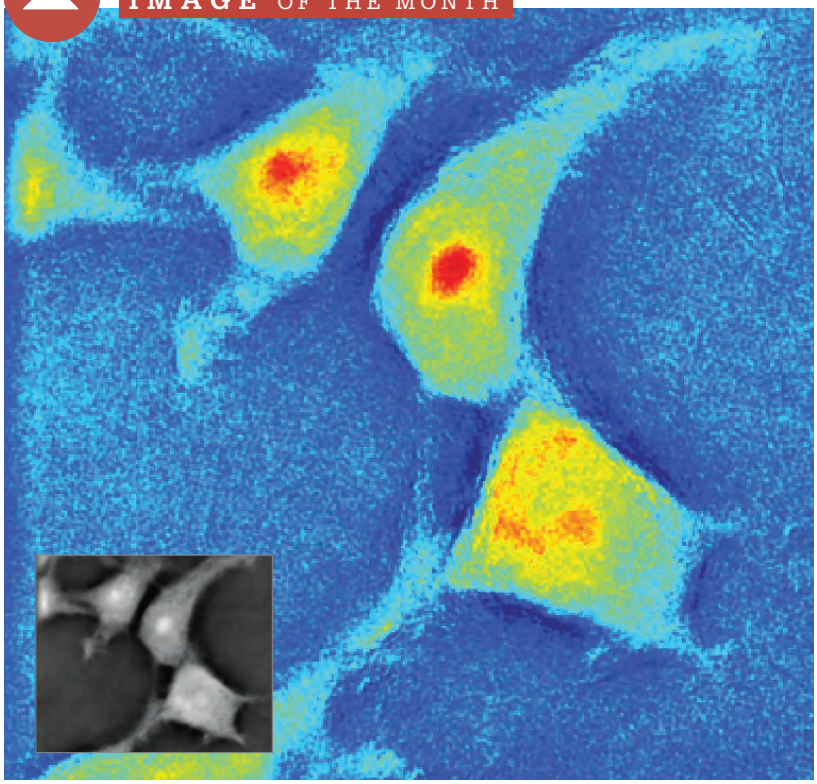
Target molecule immobilization and imprinting.

NanoMIP synthesis, affinity optimization, and linkage to conjugated polymer nanoparticles.

Confirmation of binding affinity against COVID-19 structural proteins.

SPECIAL SERIES
Infectious Disease

IMAGE OF THE MONTH



Label-Free Imaging

These images were taken using biochemical quantitative phase imaging with mid-infrared photothermal effect. They depict the label-free visualization of peptide bond-specific intracellular protein distribution (false color images) along with comprehensive morphology (grayscale images) of biological cells.

By Takuro Ideguchi, Associate Professor at the Institute for Photon Science and Technology, The University of Tokyo, Japan

Do you have a photo suitable for Image of the Month?
Send it to edit@thepathologist.com

Why Didn't They Teach This in Med School?

A series on new (and not-so-new) medical terms and diagnoses that most of us (probably) missed in training

Curated by Ivan Damjanov

Clostridioides difficile

An intestinal pathogen previously known as *Clostridium difficile*.

Medical students have been taught for many years that *Clostridium difficile* is the most common cause of pseudomembranous colitis. Not anymore! 16sRNA molecular biology studies have shown conclusively that *C. diff* is actually not a *Clostridium* at all. A proposal to rename the pathogen *Peptoclostridium difficile* prompted an avalanche of complaints from the global medical community, including the calculations of the cost of the name change. As a compromise, the bug was ultimately dubbed *Clostridioides difficile*, allowing traditionalists to continue abbreviating it *C. diff* and using the acronym CDAD, which now stands for "*Clostridioides difficile*-associated diarrhea."

Weeks 6–7

Creation of ELISA reagent for mass screening.

CPN+nanoMIP conjugate transferred to lateral flow test strip.

Weeks 8–9

Proof of concept against COVID-19.

Performance demonstration for laboratories.

Weeks 10–11

Final optimization and detection limits.

Emergency use approval.

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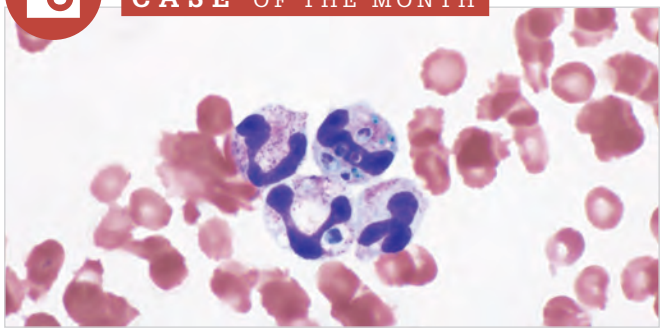
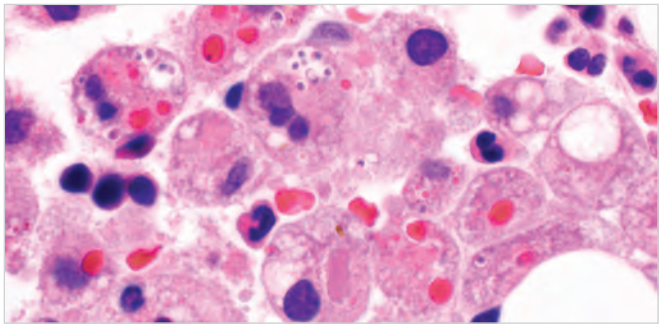
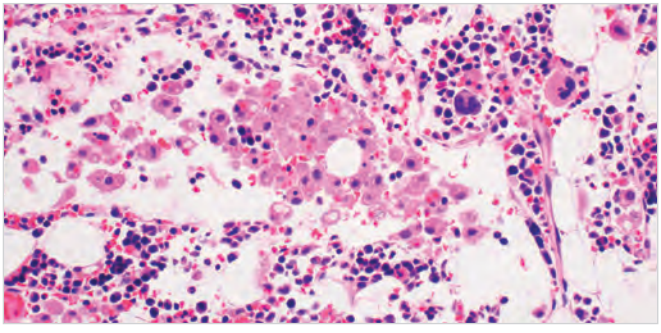
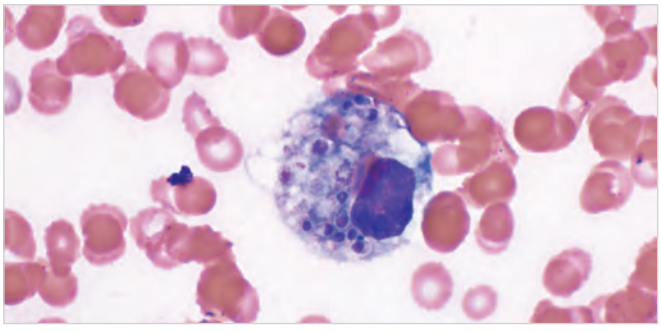
CASE OF THE MONTH

A 61-year-old female with systemic lupus erythematosus, on mycophenolate mofetil, presented with a three-week fever, hypoxia, and hypotension. Images show findings in bone marrow biopsy and peripheral blood smear..

What is the most likely diagnosis?

a) *Systemic infection by* Talaromyces marneffei
b) *Disseminated histoplasmosis*
c) *Invasive candidiasis*
d) *Visceral leishmaniasis*
e) *Toxoplasmosis*

Answer to last issue's Case of the Month…

b) *Basal cell adenoma*

Basal cell adenomas typically present as well-circumscribed, often encapsulated nodules. On smear preparations, the neoplastic population is composed of basaloid cells characterized by round to ovoid nuclei, smooth nuclear contours, granular chromatin distribution, and scant amounts of cytoplasm arranged in nests and trabeculae, sometimes with vague peripheral palisading of cells in cell clusters. The amount of matrix can be variable and can include peripheral bands of hyaline matrix around cell aggregates as well as smooth-countered hyaline globules. In membranous types of basal cell adenoma, the basaloid cells are characteristically arranged in trabeculae that are distinctly outlined by a ribbon of dense hyaline matrix material. On surgical resection specimens, these tumors often show a mixture of architectural patterns, such as solid, trabecular, tubular, and membranous patterns composed of basaloid cells with variable peripheral palisading myoepithelial cells. Immunohistochemistry for cytokeratin cocktail highlights all tumor cells, but is most notable in ductal cells, whereas the myoepithelial cells are highlighted by calponin, p63, and smooth muscle actin.

Submitted by Madelyn Lew, Associate Professor in the Department of Pathology, University of Michigan, Ann Arbor, Michigan, USA.

To register your guess, please go to http://tp.txp.to/0620/case-of-the-month
We will reveal the answer in next month's issue!

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

Oncology Biomarker Testing is Best In-House

In-house testing enables direct communication between labs and treating clinicians and ensures local healthcare quality

An interview with Michael Vieth

How – and why – do you conduct precision oncology testing at your institution?

When a clinician asks us to perform a specific test, our first step is always to identify the most suitable methods. By carrying out all testing in-house, we can adjust these methods to best suit each individual sample, maintaining regular communication with our clinicians to align testing with clinical needs. This benefits the patient because we can provide an immediate response to the treating oncologist, asking for further samples or information if necessary.

In centralized testing, specimens are sent to an external laboratory, which carries risks – for instance, logistical problems with the transit of material or communication issues because there is no direct contact with a physician. By avoiding these issues, in-house testing saves time and money. The entire diagnostic process comes from one source and we aren't left waiting for an organization to provide analyses without medical advice.

It's the rare and complex cases that really benefit from in-house testing, because those who conducted the analysis are available to discuss the results. However, different labs have different needs; to ensure that in-house testing is a sustainable option, there must be enough tests required to make the investment worthwhile.

How do different regional costs affect the choice between in-house and centralized testing?

The price that central labs charge for testing varies between regions and countries. Some labs send specimens abroad to be tested – but passing public money from one health system to another raises ethical concerns. Even if specimens are sent to central labs in the same country, they could be outside the national health system and therefore benefit external stakeholders. Different regional costs can also lead to legal issues. Regulations and side costs vary between countries – and if samples sent elsewhere are cheaper to run, that advantage must make its way back to the patient or healthcare system. If the more expensive local price is paid, then the difference could end up as profit for the central lab, which is illegal.

How does test centralization impact local healthcare?

Driving more testing through central facilities can lead to local laboratories "drying out" as knowledge, tests, patients, and money get drawn into the larger central facilities. The biggest damage that I see from losing routine cases is that you lose the ability to carry out basic science and research, which is crucial for many local facilities. Without a certain number of cases on which to demonstrate a particular testing method, it is impossible to educate people. Routine cases are an important part of the educational services of local institutions that offer medical courses – and, for complex tests that require detailed background knowledge, there's no way to learn if cases (and pathologists experienced in diagnosing them) are not available.

It's also easier to maintain tissue blocks if they are kept in-house. We have a strict tumor bank and receive up to 10 requests per day from external researchers for samples – but we always request that they return the samples without stepping down the blocks completely.

How does in-house testing make it easier to coordinate patient care?

We recently received a call from the intensive care unit. The head anesthetist had sent me a sample of bronchoalveolar lavage and told me that they suspected the patient had vaped something with an e-cigarette, so we should look for macrophages and lipid-loaded cells. We were able to react to the situation immediately in a way that would not have been possible had the sample been sent elsewhere, because it's often difficult to reach people at central labs by phone. A pathologist's second most important tool – after the microscope – is the phone, which is frequently used to retrieve information missing from cases. I often find that, especially in centrally managed labs, people fail to provide all of the necessary details about a certain case. This can be frustrating and delay diagnosis or treatment, so moving testing in-house means that you can collaborate easily and follow up quickly if any information is missing.

Another benefit comes in the form of turnaround times. Our clinicians often call at midday on a Friday and request an urgent test – for example, to determine whether a patient has a particular virus and shouldn't be allowed home over the weekend, or whether or not a patient should start immediate chemotherapy. We can usually provide an answer that same day, which wouldn't be possible if we sent the sample to a central lab via an expensive courier that might not deliver the specimen before the following week. If everything is sent externally, the in-house lab will eventually lose the expertise to carry out these urgent cases. Even if 90 percent of cases are routine, it's these few urgent ones that really prove the value of in-house testing.

Michael Vieth is Professor of Pathology and Chairman of the Institute of Pathology, Klinikum Bayreuth, Germany.

In My View

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

One More Reason to Go Digital

How performance analytics can drive quality and productivity – and optimize the bottom line

By Nathan Buchbinder, Chief Product Officer at Proscia Inc., Philadelphia, Pennsylvania, USA

Workflow automation, remote access to expertise, the ability to search repositories and get results automatically – all of these improvements and more are possible with modern digital pathology software.

But there's one advantage of digital pathology that hasn't received as much attention: new insights into laboratory performance and operations. Next-generation digital pathology platforms track analytics at the slide, case, pathologist, and laboratory levels, providing information that is central to understanding lab throughput, quality, productivity, and profitability. These analytics reveal trends over time while providing a "finger-on-the-pulse" view of what's happening in the present, potentially helping to eliminate costly bottlenecks and improve profit margins.

But how exactly do these analytics populate in real time and why are they important for improving day-to-day operations? Take a step back and consider the complex, multi-step pathology case lifecycle. Each step presents a potential bottleneck where time can be lost, creating pressure on the lab's limited resources and increasing the amount of time it takes for a patient to receive a diagnosis.

Digital pathology provides an opportunity to automatically track every step of a case from accession to archiving. Although labs previously had overview information from their laboratory information systems relating to case accession and sign-out, the movement of that case through the lab – particularly while it sits with the pathologist – is largely a black box without the performance analytics provided by a modern digital pathology system.

The routine case work performed by laboratory medicine professionals in the digital pathology platform results in a detailed case tracking log that provides real-time visibility into case status for lab managers. This visibility can help identify process pain points and sources of inefficiency throughout the case's life cycle. As changes are made within the lab to address these bottlenecks, an analytics dashboard, which centralizes aggregate information, monitors how those modifications impact lab productivity and quality. It also becomes much easier to track down overdue cases as well as report on the current status of any case wand of overall resources within the pathology lab. What's more, there's no longer a black box around what happens between case accession and archiving.

The overarching goal of performance analytics is to supply labs with the data they need to improve operations over time. Based on the trends they discover, labs can set goals, standards, and best practices to ensure that the lab processes and workflows of tomorrow are better than today's.

I recently sat down with lab leaders from a large US-based hospital system and spoke about what they saw as the potential impacts of performance analytics in their multi-site lab system. They told me that lab managers spend about 75 percent of their day, once a week, tracking the status of missing or incomplete cases and determining what needs to happen next. Then, once a month, they manually report on the performance of the laboratory, detailing how many cases each pathologist read, the types of cases they saw, the efficiency of the pathologists, how lab processes were performing, and the impact of any changes on these processes. It's a time-consuming process that likely isn't the best use of the lab's resources.

Their eyes lit up when they realized that the oversight of lab operations could be significantly streamlined with access to performance analytics, along with case tracking and global search. They could then spend more time focusing on optimizing their bottom lines and growing margins in the face of building market pressures.

In my view, analytics-enabled digital pathology couldn't have come at a better time. As pathology labs grapple with shrinking pathologist populations, decreasing reimbursements (at least here in the USA), and the rising volume of biopsies requiring diagnosis, streamlining and process improvement are more important than ever before.

Sharing Is Preparing

Medical outreach isn't only important for patient safety, it is also integral to a successful democracy

By Mark Wilkinson, Honorary Professor of Pathology at Norwich Medical School, University of East Anglia, Norwich, UK

During my histopathology training, my mentor taught me that one had not finished an autopsy until the bereaved fully understood why the patient had died. When I started applying this as a consultant in the 1990s, I realized how much comfort it gave – and how little the living knew about disease, autopsies, and death. Then, while chairing the local Research Ethics Committee, I discovered just how difficult medical researchers found the process of explaining concepts they understood to the general public. Working in a small town, I was often asked to speak about aspects of medicine and research to schools and local community groups.

Then came the Alder Hey organs scandal, in which patient tissues were retained without family consent. I was as guilty as most in the "What they don't know won't hurt them" style of paternalism. I spoke to a number of people whose relatives had samples taken and retained at autopsy. All of them seemed to have the same attitude as some of the Alder Hey parents – if they had been asked, they would have donated some organs for research (1). The resulting Human Tissue Act of 2004 required each hospital to appoint an individual who was personally responsible for delivery of a lawful service, a role I acquired at the Norfolk and Norwich University Hospital.

In 2011, the Royal College of Pathologists (RCPath) developed an outreach workshop to help students understand issues surrounding tissue retention. The two-hour "Your Body, Your Consent" workshop features a short lecture followed by group discussions (2). Pathologists who volunteer to facilitate the discussions are encouraged to allow each student to develop their own ideas and understand the validity of those expressed by others. The workshop was extremely popular and I became its lead presenter, delivering it alongside a number of other RCPath outreach sessions across universities, schools, science festivals, and other public events.

I believe that understanding pathology is central to the effective practice of medicine; my educational paradigm is that we should concentrate on the delivery of understanding, not teach facts. And it's this key principle that I applied as lead teacher of pathology at the University of East Anglia and something I still apply to all medical outreach teaching.

But what is the point of all this effort?

The cost of healthcare is significant. Northern European democracies spend about 10 percent of GDP on health; in the US, that figure is over 17 percent (3). Yet healthcare recipients have little opportunity to properly understand where the money goes. In the recent UK election, the National Health Service was a major talking point; parties offered massive sums of money without precisely setting out the future benefits. In the UK, the impossibility of delivering on the pledges made by successive governments has contributed to the devolution of healthcare decisions to local communities, and to a degree of so-called "postcode prescribing." The public understands the effects – but not the cause – of the resulting differences in care provision.

Although anyone with a sick relative can easily find promises of a cure with a short online search, few of these are free. What's more, many will not have a credible research base, some will quote incorrect statistics, and those that are factually correct are often misleading. How much emotional anguish is expended on these false hopes? And how much money does it cost? The average person with a limited understanding of the biology of disease has little chance of making an informed decision on health issues.

A basic democracy fundamentally relies upon the presence of a well-informed electorate (4). Healthcare is a huge issue in democracy and, as such, those of us who have some understanding can make a truly positive impact by sharing our knowledge in an accessible way with those who don't. Working with teenagers at RCPath outreach sessions has highlighted to me how important it is to start these informed conversations about health issues as early as possible. Not only is it inspiring for young minds, but it is also rewarding and eye-opening for the pathologists involved. I urge you all to get involved in these types of activities, wherever you can!

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Doing Our Scientific Duty

Only through research and discourse can we lead the charge against COVID-19

By E. Blair Holladay

To say that the world looks vastly different now than it did two months ago is an understatement.

When COVID-19 first emerged in the USA, few people had any idea how drastically it would change our society – let alone how we view and practice healthcare. But now, in the midst of our fight against a pandemic, we are seeing just how far we are from where we used to be – and how far we still have to go.

No one can say how or when the pandemic will end, if we will ever be truly free of the grip COVID-19 has on our society, or if it will rear its head again six or 12 months from now. All we know is that we learn more every day about this virus, and that we, as pathology and laboratory professionals on the front lines, have a responsibility to our patients to stay on top of emerging research that can help flatten the curve and ultimately stop the spread of SARS-CoV-2.

Our understanding of the virus is changing so fast it can be a challenge to keep up. New tests and platforms are quickly becoming available, fast-tracked to the public by healthcare authorities desperate to offer solutions to this crisis. Nevertheless, the challenge of disseminating necessary – and reliable – information is one we take on eagerly, because conveying the most up-to-date information as we know it is essential for the betterment of public health. From the start, the ASCP has embraced its duty to keep the pathology and laboratory community informed. When COVID-19 first started sweeping the nation, we filmed a docuseries of in-the-moment interviews with pathologists and medical laboratory scientists to showcase their tireless work in caring for the nation. We've developed a dedicated page of COVID-19 resources that we update regularly with new information. We've launched a new series of Town Hall events that provide in-depth discussions with experts on essential issues surrounding the laboratory, such as the National Testing Strategy, transfusion medicine, and multiplatform testing. And our scientific journals have published several editorials and reviews on topics affecting the laboratory right now, from the reliability of serology tests to the safety of laboratory scientists while testing for the virus.

It is this type of research and discourse that enables the pathology and laboratory community to advance the fight against COVID-19. As leaders in healthcare, it is our job to be stewards of research and information as it becomes available. And it is our job to foster the research and analysis needed to ensure that testing is effective and provides value to public health. It is on the shoulders of pathology and laboratory professionals to design and develop tests that provide accurate information so that patients and clinicians can make appropriate care decisions.

"It is our job to foster the research and analysis needed to ensure that testing is effective and provides value to public health."

As we move toward stopping the spread of COVID-19, we do so knowing that each new stage brings its own set of challenges – but we endeavor to meet and overcome those challenges step-by-step using our skills and expertise.


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Bridging the Gap

When new residents need advice in the gross room and beyond, where can they turn? Many are hesitant to go straight to faculty – and that's where pathologists' assistants can help.

Who trains pathology residents and prepares them for a career in the laboratory? The obvious answer is "pathology faculty" – those who have traveled the same educational path years, or often decades, earlier. It's true that faculty have plenty of wisdom and experience to share with their younger counterparts, but that doesn't mean that they are the only valuable contributors to residents' education. In fact, residents are sometimes so intimidated by their attendings that they hesitate to ask questions or call for help. And that's where pathologist's assistants (PAs) come in.

PAs are responsible for gross examination, specimen processing, laboratory management, and much more. Not only that, but they can also help teach residents these vital skills – and can provide a reassuring expert resource for those who may feel anxious about consulting faculty members. Without them, many residents would find themselves lost or overwhelmed. With them, the laboratory runs smoothly – and residents find their feet in an increasingly busy diagnostic environment.

OVERCOMING INTIMIDATION

PAs can provide a point of contact and reassurance between residents and faculty

By Timothy Craig Allen

As a faculty member, how do you interact with residents? How do you interact with pathologists' assistants?

My interaction with residents is multifaceted and has a strong focus on gross examination of pathology specimens. In many cases, a diagnosis can be made on gross examination and supported or confirmed by microscopic examination. I always emphasize that, although pathologists' assistants (PAs) are well-trained to gross specimens, it remains the resident's responsibility to fully understand grossing technique and correlate the gross examination with the patient's history, radiologic findings, and, ultimately, the microscopic examination. The resident may ultimately go on to hire and supervise PAs, so should master grossing technique and fully understand the reasoning behind grossing techniques. The resident must also have great respect for the PA's skills and should learn early in residency how to professionally interact with PAs.

My interaction with PAs is also multifaceted and includes direct employment responsibilities and supervision, collaboration on developing the teaching curriculum for resident teaching, oversight of the PA's professional relationships with laboratory and other staff and administration, and oversight of the PA's relationships with other pathologists.

Are junior doctors hesitant to come directly to you with issues?

As an academic pathologist, it has long been obvious that residents – particularly junior residents who are very early in their residency – hesitate to speak to me or other pathology faculty directly about their daily educational concerns unless those concerns rise to a fairly high level. I understand that completely. I was the same way as a junior resident – reluctant to reveal to the faculty my lack of understanding of what I considered the "basics." This will always be the case no matter how strongly faculty encourage communication. But that's all right because junior residents instead usually reach out to their senior residents for help.

It is important to maintain a culture where junior residents are similarly comfortable reaching out to the team's PA with their questions about grossing specimens. This outreach cannot be a replacement for a strong faculty presence in the resident's

grossing education, of course, but the resident's relationship with the PA can enhance the resident's grossing skills and, at the same time, help develop a strong professional relationship between the PA and the resident – a relationship that will lay the groundwork for successful relationships with PAs throughout the resident's career as a practicing pathologist.

How did you handle that feeling of intimidation when you were a resident?

As a junior resident, I felt uncomfortable taking what I considered simple, basic questions to my faculty members for answers – even though I held my faculty members in high regard and believed deep down that they wouldn't really mind. I just did not want to show my ignorance, even though no junior resident could have known the answers to those questions. My colleagues and I learned quickly to engage with our senior residents (and, later, junior residents turned to me as I progressed through residency). My training department did not include PAs at the time of my residency; however, had a PA been present, I would have reached out to them with some of my grossing questions. After all, the PA is specifically trained to have expertise in grossing specimens.

How can PAs help bridge the gap between learners and faculty?

Medicine is extremely dynamic today and pathology is changing rapidly. Pathology faculty are charged with understanding these changes, managing them, and incorporating them into residents' training so that trainees are fully equipped to practice in ever-changing environments. Faculty time is increasingly limited and resident training time is short. In this environment, PAs can provide valuable contributions to residents' grossing education – particularly for junior residents. These contributions do not supplant those of faculty members, but enhance the residents' grossing experience. This is why it's so important to establish a culture in which the PA is viewed as a strong team member with a well-defined role in educating residents.

As an example, I – a faculty member – work to maintain a culture where the resident learns directly from me how to gross pulmonary specimens. I am responsible for providing the resident with a detailed understanding of how pulmonary specimens should be grossed and, importantly, why we gross them that way. In this setting, the PA can help the resident with specific pulmonary specimens that may be more complicated than usual or that require advanced grossing skills the resident needs help to develop. My faculty colleagues and I are, of course, available to the residents for assistance – and we are happy to help – but that doesn't mean that residents will necessarily be comfortable coming to us, or that the PA won't be able to help in ways we cannot. The pathologist' assistant's role is not to supplant or replace our expertise, but to add to it.

What problems should junior doctors bring to faculty members? What problems should they bring to PAs?

The resident can, and indeed should, bring any and all questions or problems they encounter to the pathology faculty members. Each question or concern is an opportunity for the faculty member to educate the resident. However, practicalities limit those opportunities. And that's why junior residents can and should also reach out to their senior resident colleagues. Senior resident teaching of junior residents, including in the grossing room, provides not only great opportunities for the junior residents to learn, but also for senior residents to teach – and we know that the teacher learns more just by the experience of teaching. But practicalities limit those opportunities as well. It is here that the PA can help "bridge the gap" by supporting the team's educational responsibilities as a respected laboratory professional who can share unique expertise.

What is each person's role in the pathology teaching laboratory?

The pathology faculty member's role is supervisory, administrative, and educational. The faculty member is ultimately responsible for the entirety of the resident's education, including the development of grossing expertise. The PA's role is also multifactorial in the academic pathology department, with responsibilities that include (but are not limited to) the provision of grossing services. Ideally, the PA should perform in a culture that supports a strong, team-associated educational role so that junior residents can quickly develop strong grossing skills.

PAs have long provided grossing services in both private practice and academic settings. Academic pathology departments should develop a culture in which the PA is considered a member of the educational team and is involved in resident education in the gross room – with a very clearly defined role in supporting the pathology faculty members' grossing principles. That role enhances the team-based philosophy that is the hallmark of a strong pathology department today, supports professional respect for the PA's role, and reduces the tendency for PAs to be considered a scope-of-practice threat.

Timothy Craig Allen is Professor and Chair of the Department of Pathology at The University of Mississippi Medical Center, Jackson, Mississippi, USA.

THE IDEAL TEACHER

PAs have a key role to play in teaching residents new skills

By Marissa Spencer

Pathologists' assistants (PAs) perform a variety of tasks in the gross room. Our main role is to provide high-quality patient care by describing the gross features of surgical specimens, sectioning specimens for submission/microscopic evaluation, and creating a dictated gross report that correlates with the gross findings. A large part of our workday revolves around obtaining clinical history, specimen photography, and special workups, including intraoperative consultations, tumor triaging, mapping diagrams, and X-ray imaging. We obtain tissue for special workups, such as flow cytometry, electron microscopy, immunofluorescence, and research/tissue banking procedures. PAs maintain protocols and equipment, perform managerial duties, and take part in preparation for laboratory accreditation inspections.

But second only to patient care is the role we play in educating those around us, including pathology residents, surgical fellows, medical students, pathologists' assistant students, nurses, support staff, and even (sometimes) attendings. We are all colleagues with strengths in different areas. A PA's greatest strength is describing gross features, sectioning specimens for microscopic evaluation, and creating dictated gross reports. I think that PAs working in any environment – academic or otherwise – should take a role in educating others about this aspect of patient care, and about why gross evaluation and gross pathology are so critically important for patient outcome. In an academic environment, PAs and residents can both be learners, because the beauty of pathology is that you can learn something new every day. That said, residents can learn a lot from PAs at the gross bench. It is part of our duty to ensure that, by the end of their training, residents can independently gross cases and provide the most efficient, effective workup and diagnosis.

There is nothing more rewarding to me than seeing that "lightbulb" moment when a resident sections through a specimen after understanding the orientation correctly and fully grasping the process at hand – or even makes a differential diagnosis accurately based on what they see in the gross examination. Residents come into their training "blind" in that they know very little about gross pathology (which is only minimally discussed during their medical school training). And that can be incredibly intimidating! Past residents I have trained have confessed to me that, at times, they felt lost, defeated, and, half-jokingly, "clueless."

Naturally, most residents do not want attendings to know they feel this way! Part of our role as PAs is to help ease those fears and guide residents through their training. We can bridge the "intimidation gap" to become someone residents can confide in. It is hard to overstate the importance of establishing strong relationships with the residents you are training. As a PA, having residents that appreciate you and come to you with questions makes for optimal patient care. Why? Because residents' understanding of gross anatomy and pathology, sectioning, and submission is critically important. Many residents are intimidated by the gross room and do not want to be there – but, as PAs, our passion for gross anatomy and pathology can overcome their uncertainty and show them just how fascinating and beautiful grossing can be. We can alleviate their worries and allow them to learn without feeling overwhelmed. That passion might even make them appreciate gross pathology as much as we do. I have seen residents afraid to ask questions of PAs or attendings, and I want to encourage them to come to us – we don't bite! A resident who feels confident at the bench will feel even more confident at the scope signing out cases. It's our job to nurture that confidence. After all, the laboratory is an interprofessional environment, and we must all work together as peers to ensure the best possible patient care.

Unfortunately, not all PAs are equally eager to teach residents. Why is that? Every laboratory's goal should be an environment that fosters community and respect – but some PAs feel that teaching instead offers an opportunity for finger-pointing. For instance, what if, during sign-out, an attending disagrees with the way a gross description was worded or the way a section was submitted? Rather than take responsibility for the decision, some residents have used the PAs in their lab as a shield, claiming, "The PA told me to do it that way." Although this is not an everyday occurrence, it's one that my colleagues and I have experienced on more than one occasion. In a few cases, we have even been chastised and held responsible for errors made by residents whom we had been assisting.

Although many PAs are passionate about educating residents, issues like these create rifts between our two professions at exactly the career stage when the focus should be on building bridges. After a bad experience (or several), PAs might feel that attendings do not trust or respect them – and they will likely become less willing to help new residents. It's a problem that can only be addressed if all members of the laboratory view one another as colleagues and equals. We can be valuable resources for one another, as long as we treat each other with the professional courtesy we all deserve. And that's certainly my hope for all future relationships between residents and PAs.

Marissa Spencer is a Pathologist's Assistant at Hartford Hospital and Adjunct Professor at the University of New Haven, Connecticut, USA, and External Marketing Subcommittee Chair at the American Association of Pathologists' Assistants.

A SKILL AT RISK

Are residents losing interest in the gross room?

By John Eckman

As a certified pathologist's assistant (PA), I have worked with pathology residents at two different institutions for the past 15 years. I provide their initial onboarding, followed by one-on-one training in the gross room. After a training period, I oversee the residents working in the gross room during their surgical pathology rotations. I am also on the resident candidate interview team in our department and serve on the Resident Clinical Competency Committee.

My most meaningful impact is during their first year of residency. I emphasize to them that, although I don't replace a faculty pathologist, my job is to keep the pathologist out of the gross room until they are genuinely needed, which is why I make sure I am available for any and all questions from residents. I want them to feel comfortable asking me anything – including many (seemingly) elementary questions with which they may hesitate to interrupt a pathologist. If needed, I help prepare the resident for an encounter with the pathologist when we do call them into the gross room to discuss a particular specimen. I also try to relate to the residents during the difficult time of their training in surgical pathology, sharing with them the mishaps I have had and the criticism I have received. I tell new residents that I am familiar with the likes and dislikes of the faculty pathologists, so I can help them avoid or prepare for the few potentially unpleasant situations that can arise. I also explain that, as residents and PAs, we share a commonality: we are both grossing cases for someone else, so we face many of the same issues.

I know many PAs who work with residents, training them to gross surgicals, prepare frozen sections, prosect autopsies, and much more. We frequently share our experiences and teaching methods so that we can help each other provide the best possible training. Resident training is a frequent discussion topic at our annual conference. In addition to sharing effective teaching methods for residents, we often talk about our common experiences and some of the problems we all have. Most residents do well during training and become excellent pathologists – but some don't and, as a group, they are pushing back on work in the gross room.

Over the last several years, we have been hearing more about residency programs that require their residents to gross far less than they once did. We hear about residents seeking limitations on the specimen types they are willing to handle, claiming that some specimens offer limited educational value. This attitude has led to residents who only do a set number of each specimen type – thus missing the value of grossing a significant volume (which would allow them to encounter some less common entities). The increase in residents seeking limitations on duty hours in the gross room and, at some institutions, refusing to cover the service when the PA is away is also concerning – and seems in direct contrast to the work ethic required in pathology (and in medicine in general). Although this trend might represent some form of job security for PAs, it is worrying; some residents may not be practice-ready should they enter a setting where the practicing pathologist performs the gross exam or is responsible for oversight of gross room staff.

A recent paper from the Association of Pathology Chairs addressed some of these issues and stated concerns that the fundamental skill of performing good gross examinations was at risk due to an emphasis on non-clinical work during training. Perhaps PAs are to blame for this to some extent; after all, in many departments and laboratories, we have replaced the pathologist in the gross room. When residents see that the faculty pathologists do not gross, they may not see the gross exam as a function they will need to master before they enter practice. Some pathologists and residents seem to lose the appreciation of the expertise required to perform high quality work in the gross room as they are further removed from that process. The ability to properly examine, dissect, and sample surgical specimens is essential to patient care, particularly when dealing with cancer resections and making observations that are used to establish accurate pathologic staging. It is a privilege to perform this work and its fundamental importance to the practice of pathology and its contribution to patient care should not be overlooked or minimized. It's my hope that PAs and residents can form good working relationships, share skills, and learn from one another to preserve the vital art of the gross examination.

John Eckman is a Pathologist's Assistant at Penrose St Francis Health System/Mountain States Pathology, Colorado Springs, Colorado, USA.

LEADERS IN LEARNING

PAs are vital contributors to resident learning and development

By Cory Nash

As a pathologists' assistant (PA), interacting with residents and attendings is just as important a skill as my ability to gross. Some may consider people who work in pathology antisocial, confining themselves to the dark, windowless corners of a hospital basement. I don't see it that way. In my opinion, being social and interactive – and working to develop good communication skills – is imperative for any team member in pathology. As a PA at a large academic hospital, I interact with residents and attendings on a daily basis.

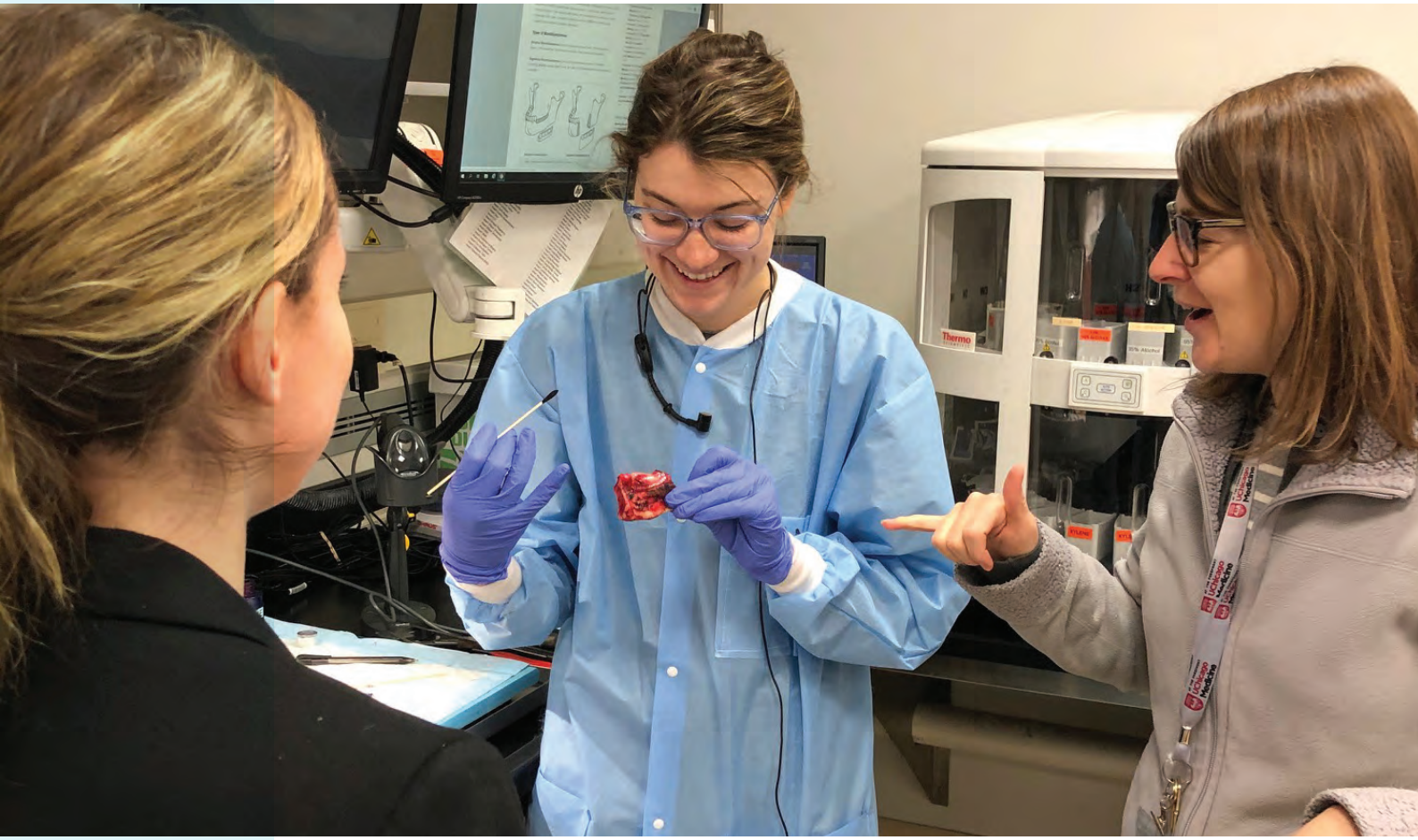
One of a PA's main responsibilities is teaching. This creates an interesting dynamic, because junior residents come into pathology as doctors – but not as "capital-D Doctors." Their training is just beginning, and they may feel a sense of incompetence when compared with senior residents or attendings. I know some junior residents initially feel intimidated by certain attendings, either because those attendings are recognized as experts in their field and the residents don't want to make a mistake in front of them, or because the attending gives off a certain aura. I am lucky to work with attendings who are very approachable; they have the skill of providing appropriate levels of constructive criticism while simultaneously holding someone accountable for their actions (and that means, "you made a mistake, so let us learn from this," rather than, "you made a mistake, so you obviously don't know what you are doing").

It is incredibly important for attendings to understand that residents are still students. They are learning every day, and it is the attending's responsibility to foster that learning. Even with that understanding, I still see junior residents who are hesitant to approach attendings with a question or concern. And not just residents – I've seen it happen with recently graduated or newly hired PAs as well. In fact, I was guilty of it myself. We are all too familiar with imposter syndrome. Only through time and experience – through making mistakes and learning from them – can we build self-confidence. Junior residents struggle with this same lack of self-confidence, and that's where PAs can step in and provide support.

Imagine a junior resident who is grossing a specimen they have never worked on before. It's a fairly complex specimen with multiple organs attached and adhesions that convolute the normal anatomy. The resident knows that they need to talk to the attending first to find out what kinds of sections they should take and how the attending would like the specimen grossed (preference in inking, sectioning, and so on). Before that resident contacts the attending, they can run that specimen by a PA, who can explain how they would approach the specimen and why. The PA can point out the different anatomic structures and the pros and cons of inking and sectioning a specimen one way versus another. In this way, the PA takes on a teaching role – and, by going over the specimen with a PA first, the junior resident can ask questions, work out problems, and potentially even discover new questions they didn't even know they needed to ask! Consider it a sort of "practice run." When the attending arrives, the resident can take the initiative and tell the attending how they think it should be grossed. The attending may or may not agree with them – but the important thing is that the junior resident stepped up and took the initiative. When you're doing that, it's hard not to build self-confidence. It cannot be overstated how important it is for junior residents to step outside their comfort zone and take on challenging specimens. As weird as this may sound, I am a big proponent of making mistakes. There are two things I always try to tell my residents and PA students. The first is that, no matter what mistake you make, someone has made it before you and it can be fixed. The second is that you need to be okay with making mistakes. Don't look at them as a negative experience, but as a positive one from which you can learn and grow.

It is no secret that PAs are experts in gross pathology. It's what we were trained in, and it's what we do every day in the lab. I say this because, if a microscopy issue were to arise, the PA might not be the best person to contact to resolve the situation – but if the problem relates to a specimen in the gross room, the first step the junior resident should take is to contact the PA. I know most PAs have faced a litany of issues throughout their careers, some of which they never thought they would run into – and some of which they didn't even know were possible. Through this wealth of experience, PAs have developed strategies to deal with these issues, and our input could be invaluable to a new resident.

Of course, certain problems should go straight to a senior resident or attending; these include situations that could directly affect sign-out or patient care. For instance, I once encountered a missing breast clip in a mastectomy specimen. An index lesion had been biopsied and, sure enough, there was a clip that we (a resident, another PA, and I) were able to find. No problem there. The imaging notes, however, stated that there was a second biopsy clip denoting benign findings. Unfortunately, the notes didn't state the size of the benign area, where it was located, or what type of biopsy clip was used. We imaged the entire mastectomy in our Faxitron, and no second clip could be identified. When we contacted the surgeon to let them know, the reply was that, according to their notes, there should have been a second clip. We made sure to assert ourselves that, despite what the notes said, our own imaging showed that there was none – and we then left it to the surgeon to decide how to proceed. Nevertheless, we still



decided to bring the situation to our attending's attention. When it came time to sign out the case, there would undoubtedly have been questions about the second clip. By alerting the attending to the situation before they received the slides, we ensured that all the proper steps were taken and that the attending could continue to have confidence in our abilities in the gross room.

The attending needs to have confidence that the gross room can function properly without direct supervision, raising a question: what is the role of each person – resident, attending, and PA – in the gross room? In the past, our roles would have been one and the same: to ensure that every patient's specimen is grossed and diagnosed in an appropriate amount of time, while at the same time providing hands-on training for residents. Unfortunately, that no longer seems to be the case. Between the increase in specimens received in the surgical pathology lab, the shortage of pathologists worldwide, and the decrease in medical students going into pathology residency, attendings are finding it harder to provide hands-on training to residents in the gross room. By necessity, they are less and less teachers of gross pathology; more and more teachers of microscopy and diagnosis.

At least in part, it's thanks to PAs that this change can happen. Their work allows attendings to focus on microscopy and teaching residents about ever-changing diagnostic criteria, IHC stains, molecular testing, and probably a million other things that I can't even comprehend. In this way, attendings and PAs work together in a symbiotic relationship whose main goal is to have all specimens grossed and diagnosed in an appropriate time frame while simultaneously training residents. In my opinion, this is how our roles should complement each other: with the PA teaching gross pathology, the attending teaching microscopy, and the resident learning from both.

We can use our roles to benefit one another and ensure the longevity of pathology. And it's important to remember that they aren't mutually exclusive; if an attending wants to offer hands-on gross pathology training, they should. We have attendings like that at my current hospital and I could not be happier. If a PA wants to learn microscopy, they should be able to learn from the attendings just like residents do. Creating a culture in which we help one another and work as a team will be of the utmost importance in the years to come. Pathology is not going to survive if one group bears all the responsibility for teaching the next generation. It is imperative that we distribute the work in the most efficient way possible to ensure that patients still receive timely diagnoses and residents still receive vital education.

Cory Nash is a Pathologists' Assistant in the Department of Pathology at the University of Chicago, Illinois, USA.

ESSENTIAL ADVISORS

PAs are a constant expert presence in the laboratory – and in resident education

By Mariam Molani

As a resident, how do you interact with faculty members? How do you interact with pathologists' assistants?

My interaction with faculty members is collegial, yet formal; my interaction with PAs is a little more friendly and easygoing. Because we rotate through numerous faculty members as we learn about various pathology specialties, we spend a limited amount of time with a lot of people. PAs are different – we interact with the same small group of PAs throughout our four-year residency. Our constant exposure to their knowledge and expertise allows us to get to know them on a deeper personal level early in our careers.

Have you ever been hesitant to bring a problem to a faculty member?

Regardless of how much I've read up on the specimen, questions often come up while I'm grossing. In the past, I've been hesitant to go to faculty with those questions. Sometimes, I'm coming back onto a surgical rotation after months of clinical rotations, so I need a refresher and to reboot my muscle memory. In these cases, it can be really embarrassing to ask faculty a question to which I'm already supposed to know the answer! Also, many faculty do not gross regularly and don't frequent specimen processing facilities. And that's where PAs come in; they are incredible teachers and have extensive knowledge of surgical specimen processing and systems management. They are skilled at anticipating issues in the gross room, histology, and specimen receiving, and they are experts at troubleshooting such issues. They also work closely with technologists and have relationships with surgeons and surgery residents – all of which is essential to effectively evaluate specimens coming into the gross room. Because we've known them since the moment we started residency, many residents feel more comfortable asking PAs questions before they bring an issue to a faculty member.

I think that, over time, I've learned to direct my questions to the person who is most intimately associated with the work or process involved. If I have a question about grossing or specimen processing, I would first direct it to a PA, whom I would consider the expert opinion. If I had a question about interpreting the histology of a particular section, I would approach faculty who signed out that type of specimen regularly.

How can PAs help to bridge the gap between learners and faculty?

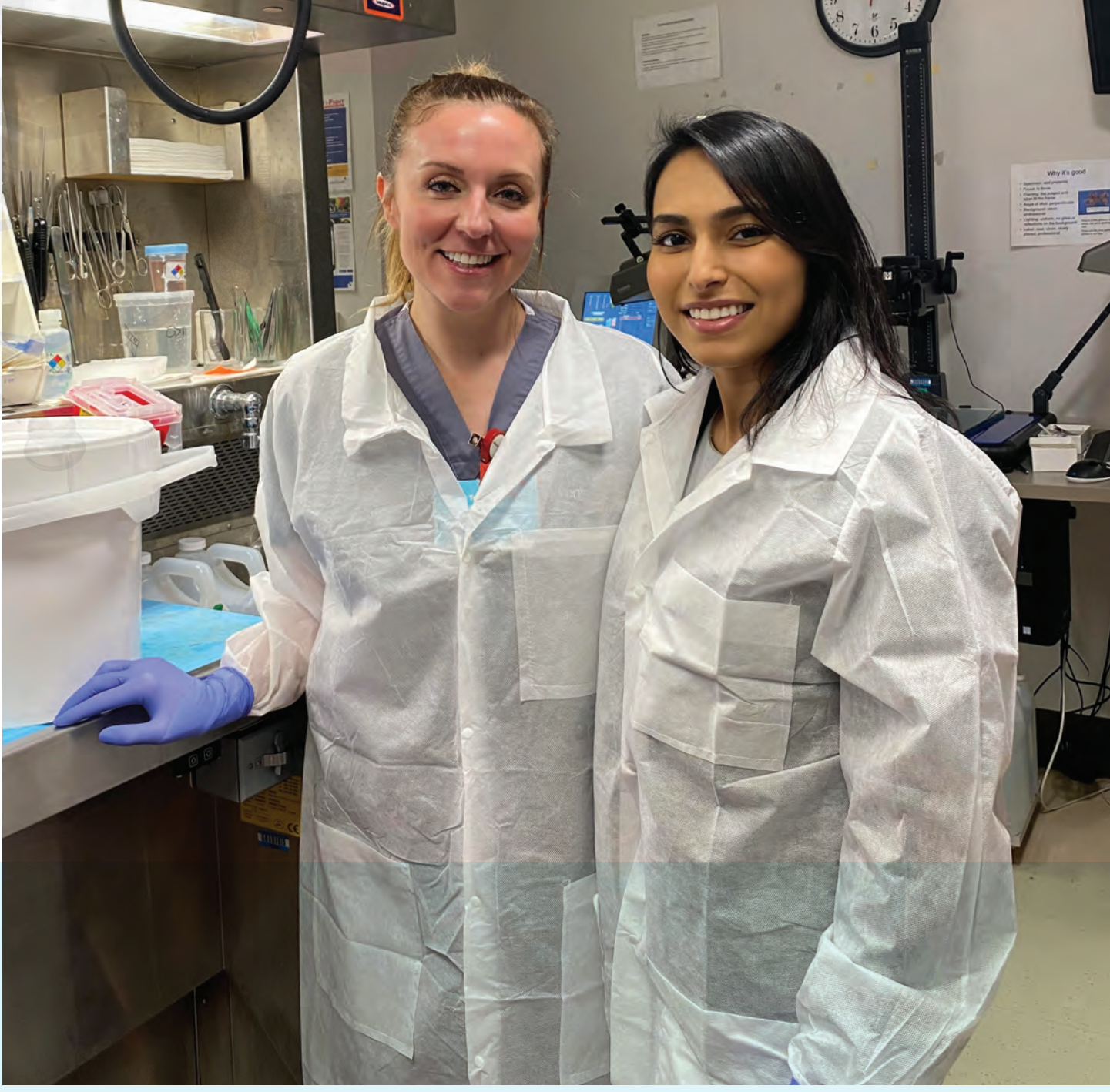
Most residency programs start residents on surgical pathology, where they learn how to gross and cut frozen sections within their first month on service. PAs are the first staff with whom residents work closely as they learn the fundamentals of pathology and tissue examination. In any given hospital, there are many more pathology attendings than there are PAs. Although pathology residents move from attending to attending on various services, they always work with the same PAs, thereby creating opportunities for strong relationships and lasting rapport. It's easy to ask someone familiar a question; it's much harder to ask someone you are just getting to know. PAs who are friendly and patient can serve as excellent teachers for residents, and the trust that residents have in such PAs limits the intimidation that we often feel with faculty.

PAs have been essential to my learning. Pathology attendings have individual responsibilities that may not allow for time in the gross room; their offices might be far away; their workload may not allow them 45 minutes to come and explain how to process a specimen. Nevertheless, I've often needed help with complex specimens – and PAs have always taken the time to explain how to orient a specimen, examine its margins, and take the right sections. I once had a mandible resection with a complex tumor; although the faculty had trouble sparing time to help me with it, a PA walked me through every step and helped me communicate with the faculty member to follow up on the case.

Sometimes, the help I've needed has been as simple as finding lymph nodes for a colectomy. As a first-year resident, I struggled to identify small nodes amongst copious fat. It was a PA who told me about Dissect-Aid, a reagent that changes the color of lymph nodes and makes them easier to find within a specimen. PAs have also been essential to me on call. They can tell me how each surgeon likes their specimens processed, who prefers gross examinations, and who prefers lots of frozen sections. By knowing the surgeon's preferences, I have been able to anticipate frozen sections and correctly gross specimens for my faculty. By being good communicators and having the patience to answer even our most basic questions, PAs can eliminate the "intimidation gap" between faculty and residents and serve as a valuable resource to residents.

What problems should junior doctors bring to faculty members? What problems should they bring to PAs?

PAs are intimately familiar with anatomy, specimen processing, and laboratory management. I would turn to a PA if I had a question about the correct technique for removing a radioactive seed from a lumpectomy, the anatomy of a Whipple, or what reagents are used to stain a frozen section. If I had a question about interpreting the



histology of a particular section, processing a very unusual case, or a margin that would affect the staging of a specimen, I would approach faculty who would receive that specimen.

What is each person's role in the pathology teaching laboratory?

A PA's role is to process surgical specimens and autopsies and to facilitate communication between specimen processing laboratories, residents, and faculty. A faculty member's role is to evaluate and diagnose patient cases and to help facilitate the processes that contribute to the preparation of cases. A resident's role is to learn medical, technical, and management skills from both the PAs and the physicians so that they can become an effective diagnostician for the patient.

In my experience, the best PAs have been excellent communicators and extremely patient with me as I made mistakes. They have also been advocates for residents and helped to bridge communication and education gaps between the gross lab and the microscope. I learned so much from them, and I don't believe I could have succeeded in residency without their support!

Mariam Molani is a Resident in the Department of Pathology at the University of Texas Southwestern, Dallas, Texas, USA, and the founder and CEO of LibraMed, LLC.

AN EXPERT OPINION

When faced with a challenge in the gross room, PAs' advice is invaluable

By Adam L. Booth

As a resident, how do you interact with faculty members? How do you interact with pathologists' assistants?

Faculty members are my guides and teachers at the microscope, sharing diagnostic pearls and criteria to prepare me for independent pathology practice. In my residency program and the majority of others, trainees "preview" cases and enter the diagnosis and report independently with graded responsibility. This process may involve ordering special stains, immunohistochemistry, additional H&E slides, and sections. Once the trainee has prepared the case, they sit with the faculty member for "sign-out" at a multiheaded microscope where the slides are viewed together. Here, direct teaching occurs, diagnostic feedback is given, and lessons are learned.

The pathologists' assistants (PAs) serve as teachers and resources in the grossing of speciments, so interaction with them largely takes place in the "gross room" – either during intraoperative consultations or while grossing surgical specimens. PAs guide junior residents as they navigate their way from simple specimens to complex resections, teaching them the appropriate steps, what to cut and where, and the necessity for particular sections to prove the presence or absence of a histopathologic finding. As a senior resident, PAs continue to serve as resources in the gross room; when I need advice regarding my approach to a complex specimen or something previously unencountered, I can speak to them for assistance.

Have you ever been hesitant to bring a problem to a faculty member?

The fabric of each pathology program is different. Faculty at my program are always available and receptive to questions, but sometimes residents (including me) still feel embarrassed to ask about things they may not know how to do. The PAs are especially helpful in that situation, giving residents an intermediary between themselves and their attendings. I believe this occurs more often with junior residents who are still getting the lay of the land and developing an understanding of personalities.

If I encountered something I didn't understand at this point in my residency, my decisions would depend on the complexity of the case and specimen(s) and impact on patient care. If I needed to ask a quick question about approach, I would speak to the PA, because they're easily accessible in the gross room with me and it's convenient. However, if I had multiple questions about a complex specimen with critical sections, I would contact my faculty for their guidance. It is always better to be humble and ask than to be prideful and make an irreversible mistake when grossing.

How can PAs help to bridge the gap between learners and faculty?

PAs can be guides and teachers in the gross room. I am sure there are faculty at programs who, for reasons either real or imagined, intimidate residents. The PA is a valuable colleague to have in the gross room in that situation. They can be present for reassurance, and ready and willing to help when needed. Additionally, their patience with junior residents is a necessity, because most pathology residents have little to no experience grossing prior to starting residency.

Recently, our department established a pathologist's assistant Master's program in which residents have the opportunity to teach some of the systemic pathology classroom lectures. This creates a great learning community even as PAs continue to teach residents in the gross room.

What problems should junior doctors bring to faculty members? What problems should they bring to PAs?

Residents should go to faculty when faced with a complex case that may impact their ability to sign out and/or provide a diagnosis or critical aspect of the report – for example, a complicated mastectomy where the margins might be in question. I know that I am likely to consult the PA if I'm having trouble orienting a specimen. Should I ink it this way or that? How should I cut? PAs are also invaluable for the more nebulous "something just does not look quite right." When that happens, I always want to get their expert opinion.

What is each person's role in the pathology teaching laboratory?

Faculty members are ultimately responsible for the case, because it's their name that accompanies the diagnosis line. In addition, they lead the laboratory, both literally and figuratively; they are often the Laboratory and Medical Directors of the lab.

In my experience, PAs play an important role in the laboratory and gross room, handling the routine challenges that arise throughout the day, such as coordinating the grossing of specimens or maintaining contact with the operating rooms. At my program, the resident and fellow on call for intraoperative consultations (frozens) meets with the head PA first thing in the morning to discuss the scheduled operations and what we can expect from them.

Residents play a unique role that spans the breadth between trainee and eventual laboratory leader. Junior residents are focused on learning proper grossing technique, whereas senior residents are expected to take on more responsibility with regard to handling problems when they arise and helping junior residents. Despite our individual roles, everyone is working on the same team to provide the best possible care for our patients.

I'm very grateful to have PAs in the laboratory to help evaluate and gross specimens – and also to teach me and my fellow residents the finer points of gross examination!

Adam L. Booth is past Chief Resident at the University of Texas Medical Branch, Galveston, Texas, a 2020/21 GI/Liver Fellow at Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA, and a Top 5 Honoree in ASCP's 40 Under Forty 2019.

Toward a Cancer-Free World

MRD testing is vital to modern cancer care – and immunosequencing is the way forward

"Cancer-free" is a wonderful phrase – but what does it really mean? For patients with leukemia, lymphoma, and myeloma, it can be a difficult phrase to trust. Why? Because even a single remaining circulating cancer cell may give rise to a relapse, triggering further rounds of treatment and their associated costs and side effects. So how can pathologists and oncologists determine which patients need ongoing treatment, which require modifications, and which may be truly cancer-free? The answer, according to Ilan "Lanny" Kirsch, Senior Vice President of Translational Medicine at Adaptive Biotechnologies, lies in minimal residual disease (MRD) testing.

What is MRD testing and how does it work?

Unlike many cancer diagnostics in use today, MRD testing is as old as oncology. We have always evaluated the extent of a patient's disease, selected our interventions, and then looked at the impacts of those interventions on the cancer. I think "minimal residual disease" is a bit of a misnomer, though, given the sensitivity of newer technologies for detecting cancer. I prefer to use the term "measurable residual disease."

Initially, measuring MRD involved nothing more than a physical exam; now, of course, we have additional tools available. At first, we developed technology that allowed us to perform a bone marrow aspirate, stain the slide, and evaluate the number of aberrant cells. Thereafter, conventional flow cytometry gave us the ability to phenotypically identify the clone of interest – and, for certain hematopoietic malignancies, we also gained serum paraprotein analysis, immunofixation, and serum and urine tests for immunoglobulin. All of those methods are still in practice today, but they are now complemented by more advanced approaches: next-generation sequencing (NGS), next-generation flow cytometry, more sophisticated imaging, and even mass spectroscopy techniques.

These newer methodologies offer greater standardization, accuracy, sensitivity, and specificity. "Why does greater sensitivity matter?" is a question I hear often. Many doctors dealing with intractable disease wish they were fortunate enough to worry about disease levels like one residual cancer cell per million normal cells. But as our treatments for cancers like leukemia, lymphoma, and myeloma continue to improve, our dream of controlling or even curing them is increasingly realistic. Event-free survival, previously measured in months, can now be measured in years. We now have many more treatment options, most of which can be tailored to individual patients' genes, so the ability to closely examine MRD and consider therapeutic options is more critical than ever.

When I report MRD levels as a number of malignant cells per million, I occasionally get calls from doctors saying, "Three per million? What am I supposed to do about that?" If they're asking whether there is still evidence of disease at that level, the answer is yes – but if they're asking whether it's actionable, that depends. Is the disease continuing to go down, is it plateauing, or is it going back up? Half a century ago, we assessed those things by crude methods and made our decisions based on the results. Nowadays, we do the same thing, but with much more sensitive tests.

How can tools like immunosequence-based MRD detection (e.g., clonoSEQ®) help?

Certain tools are now more refined, more accurate, more standardized, and more sensitive than previous iterations. The outcome? A patient who would have previously been considered "in remission" by conventional methods may no longer be "in remission" with a more sensitive assay. Now, we're able to more sensitively stratify patients who might (or might not) need aggressive therapy. Disease thresholds have previously been established to guide clinical management, but there's nothing "magic" about, for example, falling below one malignant cell per 10,000 – that just happens to be the detection limit of conventional flow cytometry. Evidence of disease at a single time point can still have prognostic significance, and trends can yield important information about disease status. Doctors may ask, "Do I need to intervene early?"

Although this has not been proven for every malignancy, it makes sense that, in general, the earlier you spot a problem and intervene, the better your chances of gaining the upper hand. This, of course, requires that the treatments available are not riskier than the disease itself.

Adaptive biotechnologies

All data, of course, must be used with caution. It's possible that, once a patient reaches a certain low-level disease threshold, MRD levels may be indicative of something else's ability to exert a positive influence on the course of disease – for instance, an immune response. MRD may be a surrogate measure for control of the disease itself.

Take, for instance, standard-risk childhood acute lymphoblastic leukemia (ALL). If patients are MRD-negative as determined by NGS at the end of the induction phase of combination chemotherapy the prognosis is excellent. In fact, a 2018 study showed an overall survival rate of 100 percent for that population after eight years (1). So now we ask: do these patients need the intensive therapy they currently receive? Could we offer treatment with a lower risk of side effects or long-term sequelae? Another example might be ALL patients in need of hematopoietic cell transplantation. Some are at higher risk of relapse than others – and MRD measurements can stratify those patients so that the ones at lowest risk can receive less intensive pre-transplant myeloablation and post-transplant intervention (2).

It's vital to determine patient needs and balance the benefits of treatment with its potential disadvantages. It's also important from a health economic perspective. If a patient is in a deep remission and does not need additional therapy, we save time and money by finding out as soon as possible. If a patient requires intensification, early discovery of that fact can prevent downstream consequences and improve outcomes. The cost of MRD testing is trivial relative to the potential costs incurred if we don't test.

What should pathologists and laboratory medicine professionals keep in mind when evaluating NGS MRD testing?

These technologies are here to stay. Nucleotide sequencing is not a flash in the pan, and immunosequencing – as applied to MRD or any situation in which cells of the adaptive immune response play a role – is one important way of learning about individual and population health and disease.

To develop and apply immunosequencing, three things had to happen: i) we needed to understand the origins of immune diversity, ii) DNA sequencing technology needed to become high-throughput and refined enough to scale, and iii) because the technology generates enormous databases, we needed bioinformatics to extract methods or results. All three of those things have occurred – and now, we have the power to mine the enormous resource of sequencing information. Now, it's time for pathologists and laboratory medicine professionals to incorporate these next-generation technologies into their thinking about health and disease.

Today, an appreciation of molecular pathology is as important as an appreciation of anatomic or clinical pathology. And that's what Adaptive Biotechnology seeks to foster – the growth of individuals as subject matter experts. The goal is for pathologists and laboratory medicine professionals to describe and interpret clonoSEQ results in tumor boards alongside imaging and histopathology, so that these results can help inform patient care. From every perspective – health economics, patient care, our overall understanding of health and disease – it makes sense for immunosequencing, and molecular pathology in general, to become a standard of care in the detection of residual disease burden for lymphoid cancers.

Adaptive Biotechnologies is the manufacturer of the clonoSEQ® Assay and provided funding for this content. The Pathologist conducted all interviews and developed all content. For technical information related to clonoSEQ®, please visit clonoSEQ.com.

clonoSEQ is available as an FDA-cleared in vitro diagnostic (IVD) test service provided by Adaptive Biotechnologies for use in B-cell acute lymphoblastic leukemia or multiple myeloma patients to detect and monitor measurable residual disease (MRD) in bone marrow samples. clonoSEQ is also available for use in other lymphoid cancers as a CLIA-regulated laboratory developed test (LDT) service provided by Adaptive Biotechnologies.

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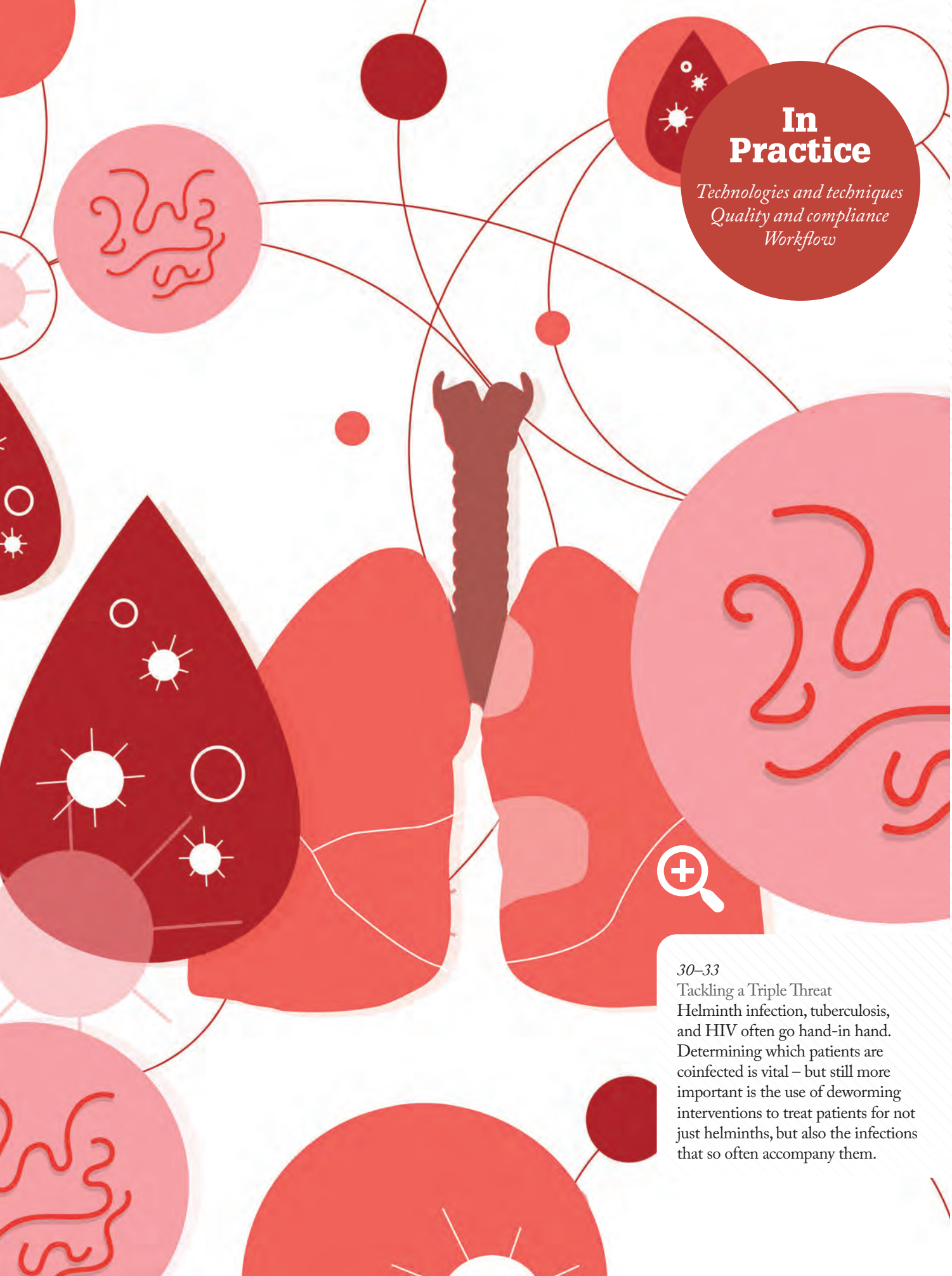

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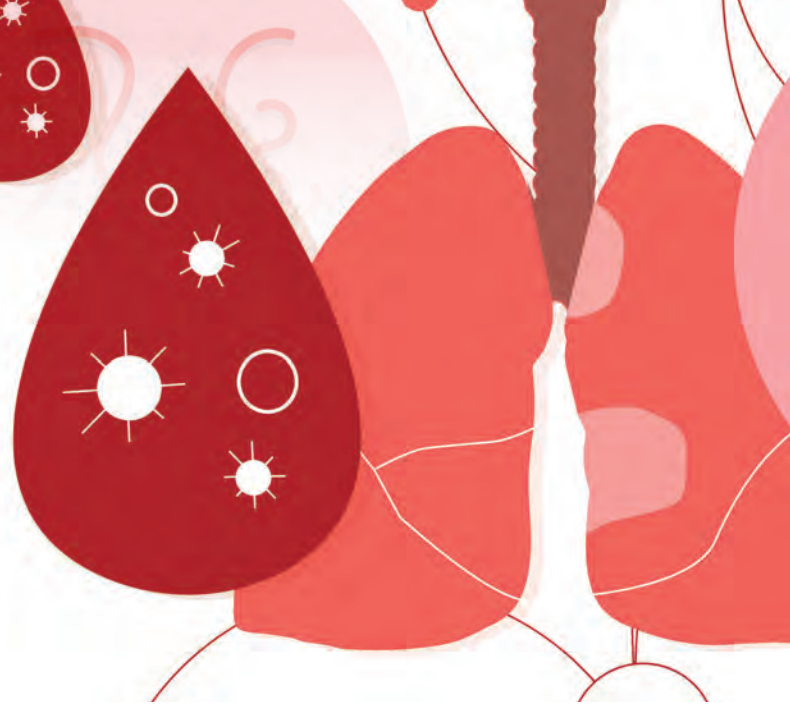

SPECIAL SERIES
Infectious Disease

Tackling a Triple Threat

The potential impact of deworming on tuberculosis treatment

By Adil Menon

Helminthic infections and tuberculosis (TB) – not only do these represent two of the most significant global public health concerns (present pandemic excepted), but there is also notable geographic and population overlap between them. Recently, researchers have begun to gain a better understanding of this geographic concordance and the commensurately high rates of coinfection. A new hypothesis states that helminthic infection may deleteriously impact the management of TB. To clarify their potential interactions, I have examined studies conducted in South Africa to establish the current state of evidence and offer a perspective on the impact that anthelmintic interventions may have on TB control.

Coinfection is common

Most TB and helminthic endemicity occur in the same settings, and coinfection is common. A broad range of countries with high helminthic burdens, such as Malawi and India, have Bacillus Calmette–Guérin (BCG) vaccination results inferior to those seen in regions with lower parasite prevalence (1). A study comparing infant BCG vaccination success demonstrated that "three months post-BCG, 100 percent (51/51) of UK infants made an IFNγ response to *M.tb* PPD compared to 53 percent of Malawian infants (1)." In the literature, the primary explanation for BCG's reduced efficacy in lower-income settings is differences in environmental mycobacterial exposure. However, another study offers a compelling alternative explanation: the interaction between helminths and TB. The researchers demonstrate that helminth coinfection correlates with diminished levels of IgM and IgG factors critical in the immune response to TB vaccination (2). The association of helminth infections with the modulation of B cell function in TB is further underscored by post-treatment data from the same paper – following successful anthelmintic treatment, the diminished levels of both IgM and IgG increased. These results support the hypothesis that BCG confers the least protection in areas with high endemic helminth prevalence because the baseline immunity in individuals living in these areas is perturbed by coinfection.

South Africa is a natural context in which to explore the interplay of helminth and TB infection. Although TB represents a serious health problem across the globe, South Africa possesses "the highest TB incidence in the world (3)". Even discounting the mortality stemming from TB/HIV coinfection, TB represents the top "natural" cause of death in the nation (4). Under these circumstances, progress toward better comprehending and responding to co-pathogens in South Africa's context may promote better health and health outcomes. Additionally, the association of helminth infections with AIDS and TB in South Africa has been recognized since the nation's independence, particularly with respect to the triple disease burden borne by the 36.4 percent of the population living below the poverty line. Despite the awareness of their potential interrelatedness, "studies of helminth coinfection with HIV/TB and their deleterious effects are lacking (5)" in South Africa to the detriment of efficient management of a significant public health issue.

A triple threat

If addressing helminth infection positively affects the treatment of TB in South Africa, it will be primarily in terms of its consequences for immune response to TB infection itself – and, due to the high rates of triple infection, its impact on HIV progression.

Let us first consider the impact of helminth coinfection on immune response to TB. Almost two decades ago, researchers suggested that, based on findings in Cape Town, "it is plausible that helminthic infections and Th2 dominance (reflected by IgE, IL-4, IL-13, IL-10) contribute to the high incidence of TB in Third World populations (6)." The potential import of Th2 bias in the context of

TB control stems from the fact that, in conjunction with innate immunity, protection against the pathogen requires "an effective adaptive cellular immune response characterized by robust T helper cell type 1 (Th1) T-cell immunity and relative weaker T helper cell type 2 (Th2) T-cell immune responses (6)." Using the tuberculin skin test as a proxy for Th1 response and, consequently, functional immune reaction to TB, a second study underscored this hypothesis by documenting an inverse relationship between *Ascaris* infections (as reflected by IgE response) and tuberculin skin test-positive status in children from high-risk, poor, urban South African communities (7); that is, the children with intestinal roundworm infections had fewer positive TB skin tests than those who were free of worms.

Though such data could imply that helminths carry a protective effect against TB, that does not seem biologically plausible. It's far more likely that "helminth exposure/infection may reduce the immune response following *M.tb* exposure (7)." If this is the case, then there are two potential advantages of deworming:

1. *The possible reversal of the demonstrated Th2 bias*. A Th1-focused immune response is well-established as critical for an optimal immune response to TB.
2. *Improved diagnosis*. According to the government of South Africa's Western Cape Province, "testing for children is done using skin tests and chest X-rays (8)." Given the high prevalence of pediatric TB in South Africa and the methods used to diagnose it, there is enormous potential for undiagnosed TB due to helminths – especially if Western Cape Province's diagnostic approach is the norm throughout the nation.

But an exploration of the interplay between helminth infection and tuberculosis treatment in the South African context is incomplete without considering a third pathogen: HIV. South Africa ranks among the worst-afflicted countries in the world for both HIV infection and TB. Half of all new TB cases in South Africa are diagnosed in HIV-coinfected patients (9). Given these statistics, any factor that worsens HIV prognosis and progression almost certainly plays a deleterious role in TB prevention and treatment.

The high prevalence of coinfection between helminths and HIV is well-established in South Africa. One study demonstrated a 24.7 percent HIV/helminth coinfection rate, with 42 percent of these patients hosting *Ascaris lumbricoides*, the "large roundworm" (10). Crucially, the study authors observed a statistically significant association between a CD4 count below 200 cells/μL and a helminth infection.

A second study bolstered the results of the first and addressed a number of its weaknesses by using both egg observation and serology. The researchers in this second group determined that HIV immune responses are impaired by helminth infections in certain susceptible groups of individuals, particularly those who are infected enough to excrete worm eggs and exhibit high serum IgE concentrations (11). Individuals in this subgroup were marked by eosinophilia, and the HIV-positive subjects with high IgE had close to three-fold higher viral loads than those with low IgE. Immune activation is known to be associated with a temporary increase in HIV viral load, even among otherwise well controlled patients; this means that a less robust antiparasitic immunological milieu (as reflected by a low IgE phenotype) could play a role in mitigating this aspect of HIV viral infection.

The worm in the apple

Both eosinophilia and high IgE are classic Th2 responses commonly induced by helminth infections – so there are a number of biologically plausible theories as to how helminth coinfection worsens HIV prognosis. One such theory is that eosinophils increase the number of activated cells that are vulnerable to HIV infection. (How? In large part through their ability to prime CD4 cells, rendering them susceptible to HIV infections.) Findings consistent with this theory have existed in the literature for decades (12). The collective findings from the high IgE groups also concur with the suggestion that chronic helminth infections in adults disrupt

"The immunological milieu induced by helminthic infection worsens the body's ability to respond to HIV and TB."

peripheral T cell populations (11). This link is underscored by the fact that IgE – and no other immunoglobins – has been "inversely related to helper T cell and suppressor/cytotoxic T cell numbers (13)." Taken as a whole, these results strongly support the idea that the immunological milieu induced by helminthic infection worsens the body's ability to respond to HIV and, commensurately, to TB.

There are many good reasons to argue for the value of deworming as a stepping stone to more robust TB treatment – primarily its impacts on immunology of both TB and HIV, but other potential advantages as well. The frequency of coinfection offers not just the opportunity to improve treatment of tuberculosis by undercutting its partners, but to directly and simultaneously treat multiple conditions with the same agent. For instance, research has demonstrated the enormous anti-mycobacterial treatment potential of the avermectin family of anti-helminth agents. In the course of one study (12), every avermectin that was evaluated showed mycobactericidal effects, "reducing initial bacterial viability up to six orders of magnitude (12)." What makes these results all the more exciting is the fact that all avermectins tested showed promising bactericidal activity against the multidrug-resistant strain of *Mycobacterium tuberculosis*, suggesting that we might be able to add avermectins to the small pool of agents we can use to treat TB patients with drug-resistant disease. These medications are especially practical therapeutic options because of the low exposure (AUC/MIC ratios of 10 to 15) needed to achieve clinical effect. A further attraction is that "the specificity of avermectins for mycobacteria would be ideal for selectively targeting the pathogen while minimizing deleterious effects on resident gut flora after oral administration (12)." Ultimately, the authors conclude that "the promising 2 to 8 µg/mL range of MICs for avermectins in liquid cultures is comparable to that of second-line TB drugs, ranging from 1 to 25 µg/ml against *M. tuberculosis* (12)."

This work is the first demonstration of the antimycobacterial activity of avermectins and, consequently, the potential for simultaneous co-treatment of helminths and TB. Potentially adding some field-based credibility, a follow-up study – once again examining *Ascaris* in urban South African children

– described the anti-mycobacterial potential of anti-helmintic agents as a plausible explanation for its seemingly contradictory results (13). It is possible that sufficiently rapid deworming can clear mycobacterial infection before the appropriate Th1 response reaches culmination – though, of course, further study is warranted.

Finally, beyond their direct positive impacts, deworming programs have often served as entry points to reconstructing and bolstering public health. They provide models, generate confidence in interventions, and help to facilitate partnerships between communities and academic institutions. Intervention research in an informal settlement in Cape Town, for example, demonstrated that a successful school-based deworming program can play a key role in convincing parents and teachers to get involved in health care delivery. All those involved in the intervention, including the children, came to appreciate that health education focused on local problems is beneficial. The researchers also demonstrated – though without fully elucidating why – that synchronized mass deworming programs, especially in densely populated informal settlements, can contribute to overall health. It's findings like this that have motivated scholars to consider investment in deworming as a practical and affordable way to help "reduce the incidence of infection by HIV and tuberculosis, slow down the progression of the diseases they cause, and improve the efficacy of vaccines against HIV/AIDS and TB (15)."

Many birds, one stone

At least in South Africa, strong evidence exists that helminth infection may exacerbate TB. Most critically, parasitic infections and mycobacteria engender two divergent immune responses. Consequently, a person mounting an appropriate response to a helminth infection undergoes an immunological shift that leaves them increasingly vulnerable to TB. Compounding this situation, the Th2 predominance of the immunologic response to TB can render it more vulnerable to HIV progression. With these immunological consequences in mind, the value of deworming as a potential positive factor for TB control seems clear.

Its potential is only enhanced by emerging evidence that anti-helminth agents may also treat mycobacteria with similar efficacy to second-line anti-mycobacterial drugs – and, of course, that deworming has significant positive effects on overall community health. As South Africa has long believed, and the literature increasingly indicates, helminth infection is very much an equal partner in a deadly triad alongside tuberculosis and HIV. This research, along with corroborating evidence from other African nations, strongly indicates that deworming may soon be a key component of treatment not just for helminths, but also for TB.

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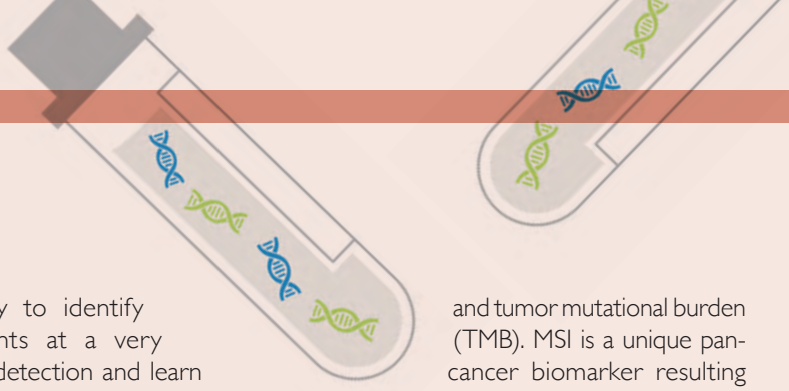


Liquid Biopsy Insights into Cancer

The amount of time between the arrival of a new technology and its deployment in the diagnostic laboratory is shrinking. Take immuno-oncology (IO), for example – this powerful new approach harnesses the body's own immune system and has quickly become one of the most promising approaches to cancer treatment. Yet given the growing number of guideline and clinical trial biomarkers that can be explored, tissue sample availability is becoming a limiting factor. Liquid biopsy provides a noninvasive alternative, not subject to tissue limitations, to gain access to the mutational landscape of tumors.

As molecular pathology assays aim to cover a growing number of guideline-recommended and emerging biomarkers, existing assays that interrogate a small number of variants can now be consolidated onto a single panel, facilitating comprehensive genomic profiling (CGP) of multiple actionable biomarkers and key investigational IO biomarkers through next-generation sequencing (NGS). Covering these needs with a combination of revolutionary sample collection and consolidated testing is key, especially in current times with infectious threats adding another layer of challenge for oncology patients.

Enabling CGP from liquid biopsy sounds like the perfect answer; the approach is noninvasive and enables repeat sampling. However, it raises questions about the feasibility of analyzing a variety of tumor variants and genomic signatures that are circulating in the blood with high sensitivity. We spoke to two experts working in molecular pathology and translational genomics to explore the merit of liquid biopsy to identify tumor insights at a very low limit of detection and learn about the early success of Illumina's comprehensive RUO assay, TruSight™ Oncology 500 (TSO500) ctDNA.

Multiple markers

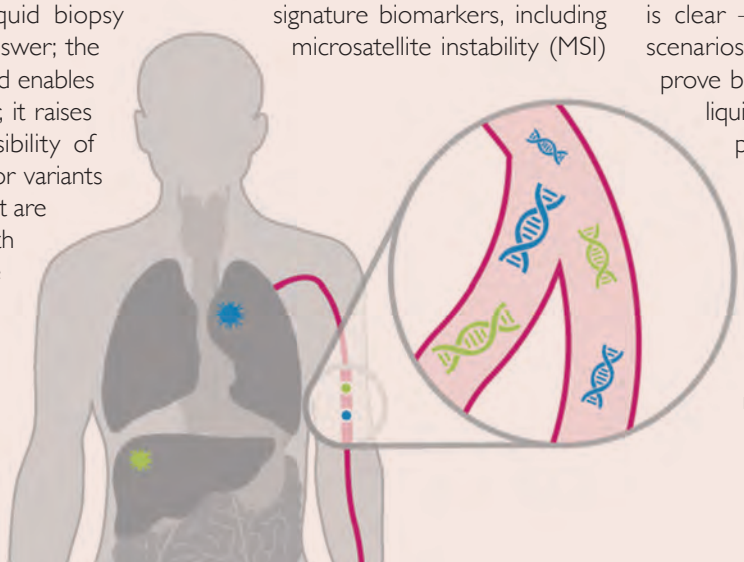
"When it comes to sequencing and beyond, the days of the single-focus assay are numbered. There are now hardly any tumor entities in which we only look for one marker," says Wilko Weichert, Professor of Pathology and Chairman of the Institute of Pathology at the Technical University of Munich, Germany. Larger panels, with a variety of markers enabling the identification of multiple potential driver alterations provide a more detailed prediction of therapy response and will allow clinicians to select the most appropriate therapy according to individual tumor characteristics. "The choice between targeted panels and comprehensive assays depends on intended use – but for anything from exploratory biomarker discovery to composite biomarker testing, it's important to have multiple markers consolidated into a single assay," says Stephanie Hastings, Manager in Assay Development, Translational Genomics at Q^2 Solutions, USA.

The TSO500 ctDNA assay is one such highly multiplexed assay that involves the detection of small variants, copy number variations (CNV), fusions, and key genomic signature biomarkers, including microsatellite instability (MSI) and tumor mutational burden (TMB). MSI is a unique pan-cancer biomarker resulting from defective DNA mismatch repair, which indicates predisposition to mutations. TMB measures the number of mutations within the coding sequence of the tumor genome. Combining multiple genomic aberrations provides a highly personalized assessment.

However, pathologists routinely work with solid tumor tissue samples – so why would they use liquid biopsy? "There are two main advantages of using liquid samples. Blood is readily available, and you can collect it via a minimally invasive procedure, repeating several times if necessary," explains Weichert. "It's also accessible in almost every patient – even those in whom you can't reach the lesion by traditional biopsy due to an increased risk of side effects, such as lung cancer patients with emphysema."

Another factor to consider is the biology of the two different sample types. Although solid tumor samples might correlate well with histology and cellular phenotypes, they represent only a small, localized primary tumor profile. In contrast, multiple metastatic lesions might all shed DNA into the bloodstream. The subsequent liquid biopsy sample provides a comprehensive patient tumor profile that could be more predictive of therapy response than information from a single tumor site.

The potential value of liquid biopsy is clear – and Weichert sees several scenarios where a liquid sample could prove beneficial. "I believe we will see liquid biopsy used to follow patients, particularly because it's easier to obtain blood sequentially than tissue. For example, you can measure ctDNA in the blood to check for cancer recurrence or monitor changes in molecular profiles to detect whether resistance mutations have occurred."



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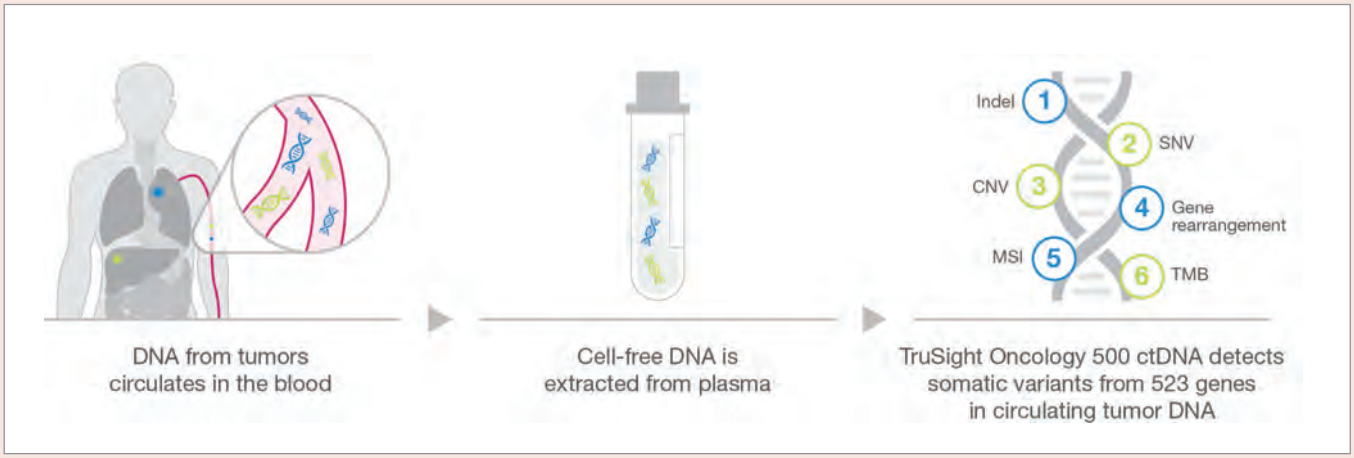

Effective capture

"The real challenge of working with a liquid biopsy sample is its potential for low stability; lysis of white blood cells can cause genomic DNA to spill into the plasma fraction," says Hastings – and such contamination could potentially conceal the targets of interest in the blood sample.

Therefore, appropriate collection tubes, preanalytical considerations, and using the most effective workflow are key to capturing low-frequency molecular alterations present in cell-free DNA (cfDNA). "When it comes to sample quality for liquid biopsy, the way that blood is collected is crucial – for example, the tube type used can affect the level of stability," says Hastings. "Once you've isolated the ctDNA, the way in which you quantify the amount of material you have is also important for assay performance." It is critical to use an electrophoretic quantification method, such as Fragment Analyzer or TapeStation, that can specifically measure the cfDNA fraction and exclude high-molecular-weight DNA contamination. Fluorometric methods are not recommended because they quantify all species of DNA sizes contained in the sample, which could potentially overestimate the amount of cfDNA and fail to create a robust library (1).

From a library preparation perspective, using hybrid capture-based target enrichment chemistry enables users to generate results from liquid biopsy samples with very high sensitivity. Hybridization probes have tolerance to capture targets even when mutations exist in the hybridized regions and can cover the span of the entire gene sequence. In comparison, amplicon-based chemistries only amplify a subset of the fragmented DNA due to the possibility of break points between primer binding sites. This also makes amplification of novel fusions challenging. Hybrid capture is more versatile than amplicon-based chemistry and can be used to detect SNVs, indels, CNVs, fusions, and other structural changes with higher accuracy. "A hybrid-capture approach is therefore more robust than amplicon because of this feature; you will gain higher sensitivity by having a purer sample for analysis."

Working in collaboration with Illumina since 2018, Hastings has been able to evaluate the assay chemistry of TSO500 ctDNA that has enabled the detection of low allele frequency variants such as EGFR L858R, MYC indels, and NTRK2 fusions. "Early access to the pre-released version has allowed us to comprehensively evaluate its performance – and, to date, we have analyzed over 1,000 samples using the TSO500 ctDNA assay."

Liquid biopsy's future

Liquid biopsy is still very much an evolving application – and its increased use in clinical trials could accelerate adoption. To that end, Weichert believes that a combination of both tissue and liquid samples should be used wherever possible in these scenarios; "We need even more directly comparable data that indicate whether liquid samples are more predictive than tissue samples, as we envisage they might be."

Whether in the diagnostic laboratory or in clinical trials, liquid biopsy is a fast-moving field that directly feeds into the future of precision medicine. TSO500 ctDNA is proving to be an effective research tool for early-access users, harnessing easily accessible, reproductible tumor content to deliver comprehensive information across 523 cancer-related genes. Hastings certainly believes in the potential of the platform, given that Q^2 recently performed analytical validation of TSO500 ctDNA.

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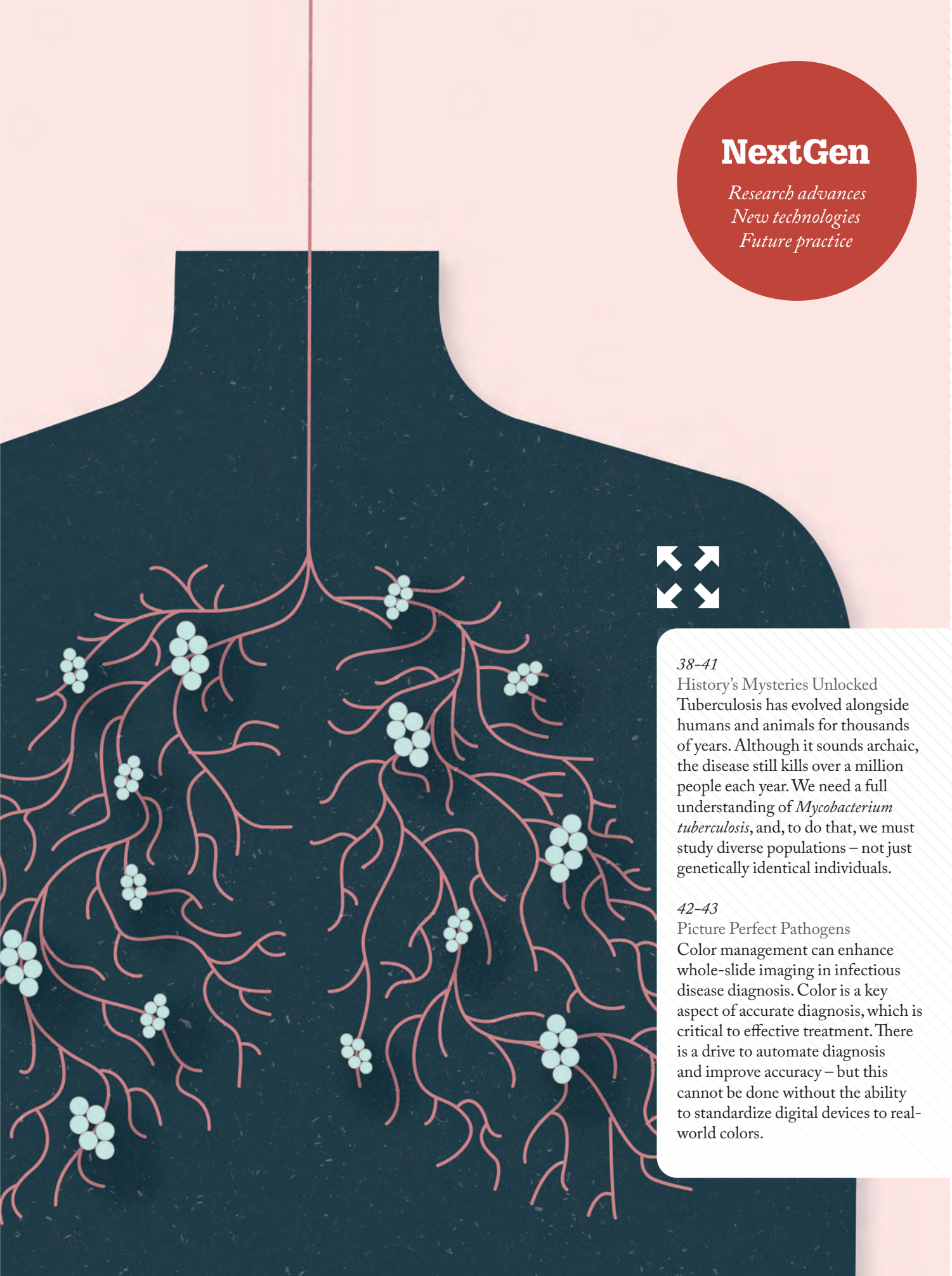

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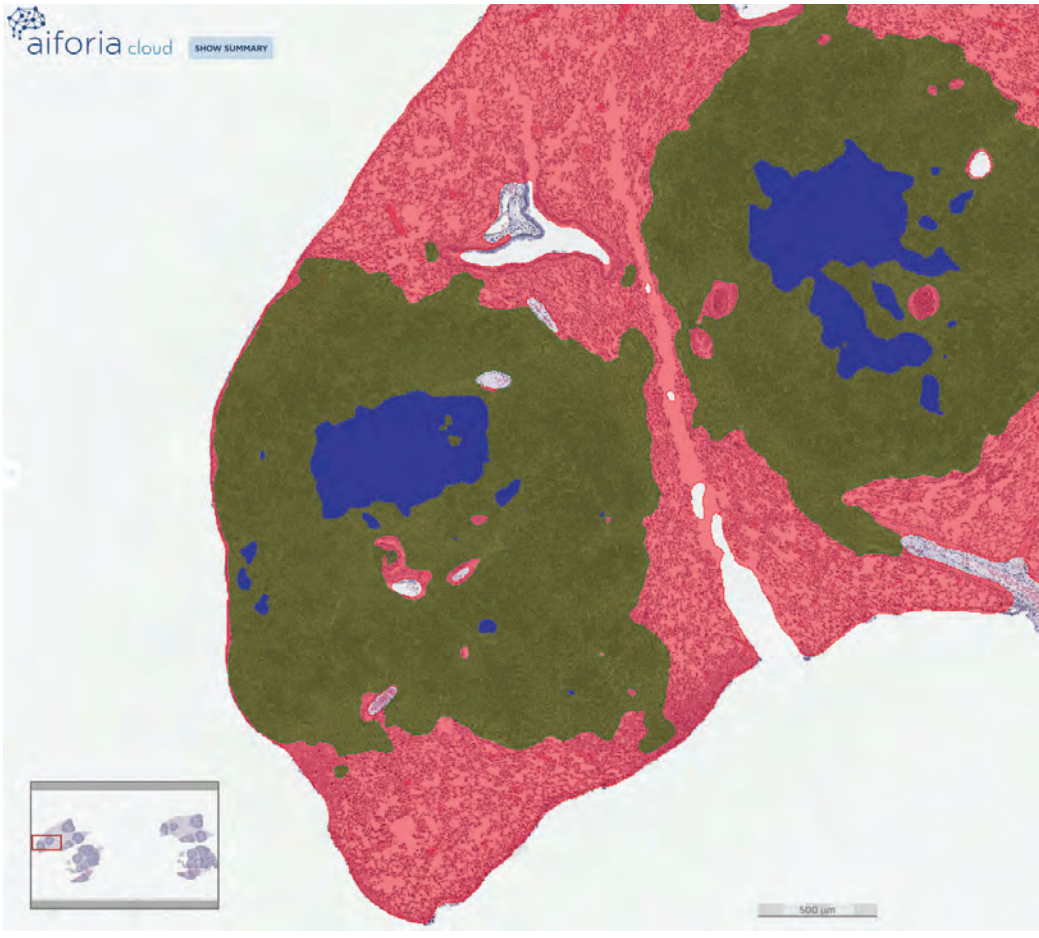
SPECIAL SERIES
Infectious Disease

History's Mysteries Unlocked

Using animal population models and artificial intelligence to understand tuberculosis

By Thomas Tavolara, Muhammad Khalid Khan Niazi, Thomas Westerling-Bui, Metin N. Gurcan, and Gillian Beamer



Tuberculosis (TB) may sound like a disease of the past, but this is a grave misconception. Although TB is not common in the developed world, it causes more deaths than HIV/AIDS or malaria, worldwide. Further, antibiotic resistance has increased, making it harder to treat some TB patients. TB is caused by a fascinating bacterium; *Mycobacterium tuberculosis*. Over thousands of years, *M. tuberculosis* adapted to life in the human body, developing abilities to persist asymptomatically in resistant hosts and cause lung-damaging inflammation in susceptible hosts. This inspires our research question: What controls host susceptibility? Nine out of 10 people infected with *M. tuberculosis* do not become ill. They don't show symptoms, they don't need treatment, and they aren't contagious. What are the differences between the majority who never become ill, and the small fraction who do develop active TB? We seek explanations for these differences from the perspective of host genetics. We hypothesize that genetic differences in hosts can help explain differences in susceptibility to *M. tuberculosis*. More specifically, we think increased susceptibility reflects genes that increase inflammatory signaling when cells detect *M. tuberculosis* bacteria in the lungs.

A model population

To address our hypotheses, we use the Diversity Outbred mouse population. The DO population has as much genetic diversity as the human population and like individual people, each DO mouse is genetically unique. We are modeling (to the best of our ability), the genetic diversity of the human population and the range of responses that humans exhibit when infected with *M. tuberculosis*.

Experimentally, we can identify the genetic makeup of DO mice who are susceptible (or resistant) to *M. tuberculosis*. By studying how DO mice respond to *M. tuberculosis*, we can locate regions of the genome (i.e. specific DNA segments) that are associated with responses to *M. tuberculosis*. This is a mechanistic approach to understanding TB. We are also using information from DO mice to build artificial intelligence (AI) models that can classify (diagnose) and predict (prognose) outcomes to infection. First, we identify the features that correlate with susceptibility or resistance to *M. tuberculosis* in the DO studies. Then, we test whether the features can accurately diagnose or prognosticate the host outcome in a blinded fashion on new experimental data. We are trying these approaches with many data types: genomic, gene expression, protein biomarkers, capacity to restrict *M. tuberculosis* growth, etc. Now, we are applying AI to lung histology -

granulomas, cellular and tissues responses - any visual manifestation of the host response

Our work

We found the DO mouse population response to *M. tuberculosis* similar to that of humans. Some individuals are very susceptible – becoming ill within a few weeks – whereas others are quite resistant and appear healthy for months or even years. These widely different outcomes occur in adult mice of the same age given the same amount of bacteria at the same time on the same day. All mice live in the same environment. The one thing different about each mouse is its genetic background – so the different

"The degree of density and distribution of nuclear debris in lung tissue may be a predictive factor for susceptibility."

responses can be explained by different sets of genes that control the host's response to *M. tuberculosis*. Using this mouse population, we want to identify the genes and pathways that lead some hosts to develop early and severe lung-damaging inflammation. If we can identify a cause of lung-damaging inflammation, we may be able to target a pathway for treatment in patients with active tuberculosis. We also found that certain combinations of molecular biomarkers better discriminate which DO mice are susceptible to pulmonary tuberculosis than single biomarkers. This too links to some underlying genetic phenomenon we hope to trace and exploit so that we can identify which mice will be susceptible to *M. tuberculosis* infection.

Many genes are involved in a host's overall resistance or susceptibility to *M. tuberculosis*. We think we are discovering that lung-damaging inflammation and *M. tuberculosis* restriction are controlled by different pathways. I am surprised by that preliminary conclusion, because I had previously imagined that infection and inflammation were somewhat of a chicken-or-egg situation – nearly impossible to tease apart. With the DO mouse population, we can start to differentiate between the host pathways that cause lung-damaging inflammation and the host immune responses that restrict bacteria. From an AI perspective, we may also conclude that the degree of density and distribution of nuclear debris in lung tissue may be a predictive factor for susceptibility.

In the future, we plan to undertake detailed studies of the specific genes and pathways that cause TB in susceptible individuals. A greater understanding of disease will lead to better individual risk assessment and prognosis, as well as improved vaccination strategies, therapies, and monitoring. We will also use AI to tease apart specific imaging biomarkers indicative of different disease susceptibilities. We may be able to map between different information domains, predicting pathology from gene expression data or simulating tissue given a specific mouse genotype. Through these studies, we will gain a much better understanding of the relationship between genotype and phenotype.

We are also using the DO mouse population to identify and validate biomarker signatures that can predict disease outcome before infection occurs. We may have a translational advantage because we are working with a genetically diverse population – which is why I'm so grateful to the investigators who had the vision and capacity to push the development of the DO mouse population. Their service to science is immense.

A helping hand from AI

Most pathologists recognize patterns of microscopic change (lesions); group the lesions into clinically relevant states (diagnosis); attempt to predict host outcomes (prognosticate); and integrate clinical or research data in context to understand how disease occurs. AI can help pathologists perform the first three tasks consistently and efficiently.

"What was incredible with our 'bag of tricks' method was that the AI automatically extracted a feature clearly interpretable by any pathologist."

The premise of the latest AI trends is that, given enough examples of a feature, such as a granuloma in a lung section, AI can also accurately classify that feature. Further, an AI system can also learn to reliably classify the disease state of a patient given enough prior examples of that disease in other patients. Like pathologists, AI can use many different data sources – hematoxylin eosin-stained sections to clinical laboratory data and constitutional symptoms. The concept of mapping a domain of information (imaging or otherwise) to a specific outcome is called *classification*. When a pathologist performs this process, it's called diagnosis.

A (brief) overview of AI in tuberculosis

We are not the first group to use AI tools for TB research. Many publications demonstrate methods to detect *M. tuberculosis* bacilli in digital images of sputum samples. These methods generally follow two basic steps – segmentation of *M. tuberculosis* bacilli and classification of segmented objects as bacilli and non-bacilli.

Segmentation generates potential candidates for bacilli by examining the color of the pixels in the image. In lung tissue sections from *M. tuberculosis* infected DO mice, Acid-fast staining colors the bacteria red and the rest of the tissue has a blue counterstain. Thus, red pixels are simply classified as part of bacilli, and every other pixel is classified as background. Specific methods for partitioning red pixels from the background include thresholding, clustering, artificial neural network approaches, Bayesian segmentation, and fuzzy segmentation. Once potential bacilli candidates are generated via segmentation, they are filtered for false positives (due to small artifacts in the image) through feature extraction and subsequent classification. Features extracted from the bacilli candidates include perimeter, eccentricity, area, Fourier descriptors, and Hu's moments. Classifiers on these extracted features include support vector machines, Bayesian classifiers, and artificial neural networks.

In addition to detecting bacteria, AI tools may be used to diagnose TB in chest radiographs or other types of patient scans. These methods generally use deep learning to classify radiographs or scans as active pulmonary TB or healthy. This classification is sometimes preceded by a segmentation of cavities characteristic of severe lung damage. These AI methods require a ground truth segmentation prepared by a radiologist.

Though these methods are accurate in diagnosing active pulmonary TB, they don't predict who will develop the disease, or to what degree. We envision results from studies in *M. tuberculosis* infected DO mice may translate to a better ability to identify who is susceptible to TB, when he/she will develop TB, and the severity of disease. To this, we need AI tools to turn the visual information from the granulomas of *M. tuberculosis*-infected DO mice into quantifiable data suited for statistical analyses and machine learning. In essence: AI to detect, localize, classify, and quantify visual patterns into actionable information.

How we use AI

In pursuit of these goals, we use AI in several projects to automate mundane or time-consuming tasks, model complex imaging data, and discover underlying phenomena in histopathology images. We use AI to automatically detect and quantify features of interest within lung granulomas. That can be straightforward, such as counting macrophages, which is easy for one granuloma, but not feasible to scale up. It's difficult for a pathologist to crank through 1,000 slides. High-throughput quantification is easy with AI but challenging for your

pathologist…unless you are trying to get them to quit.

As an example, we've made an algorithm, using the Aiforia platform, that can count foamy macrophages within granulomas. Hundreds or thousands may be present in a single granuloma. It's impossible for a person to count thousands of macrophages in thousands of sections from the over 1,000 mice we have. I wouldn't eat or sleep; I would be chained to my microscope (and my thumb would hurt from pressing my counter so many times). I've turned to AI to extract foamy macrophage numbers that we use in downstream analyses. AI spared me a tedious task and saved my thumb.

Beyond granulomas, we've developed AI tools to automatically segment necrosis, lymphocytic cuffs, macrophage-rich regions, neutrophil-rich regions, infected tissue, and healthy tissue in hematoxylin- and eosin-stained lung sections. In these tasks, we are interested in geographical relationships between the location and distribution of different cell types, and anatomical structures that may indicate susceptibility to TB. And though we already have robust method to localize all nucleated cells (H&E) and to segment *M. tuberculosis* bacilli (acid-fast), there's no way I could enumerate all those relationships. So again, AI can automatically identify those anatomic sites, localize cells or bacilli, compute complex relationships between and among these regions and their cells, and turn that into something useful, like predicting the susceptibility of that DO mouse.

Recently, we developed an exciting AI tool (which I refer to as our "bag of tricks") that automatically identifies imaging biomarkers for susceptibility using only lung histopathology images and the susceptibility category assigned to each DO mouse. More specifically, we have focused on the "supersusceptible" DO mice that develop pulmonary TB within eight weeks of infection. Usually, AI tools require that pathologists manually annotate diseased areas on a slide to provide a ground truth. However, here we developed an AI tool to diagnose supersusceptible DO mice using only the category label and the lung histology image. No manual annotation was needed. Further, what was incredible with our "bag of tricks" method was that the AI automatically extracted a feature clearly interpretable by any pathologist. In this case, the feature AI used to classify supersusceptible DO mice – which we are dubbing an "imaging biomarker" - corresponded to pyknotic nuclear debris. This is useful for our TB research, but also has broad implications for the field of computational pathology, in which diagnosis labels at the slide level can yield interpretable imaging biomarkers, discover new image features of diseases, and help to validate existing clinical biomarkers.

Now that we can comfortably delegate to AI, what should pathologists do? Our brains are our best asset. Our most important contribution to research or patient care is thinking, integrating information, solving complex problems, and asking the next set of questions. What do the observed changes mean in biological context? What did we learn about the pathogenesis of disease? Is this a new disease? That's what I want our jobs to be.

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SPECIAL SERIES
Infectious Disease

Picture Perfect Pathogens

Color is vital in a field where special stains are key to diagnosis

By Richard Salmon

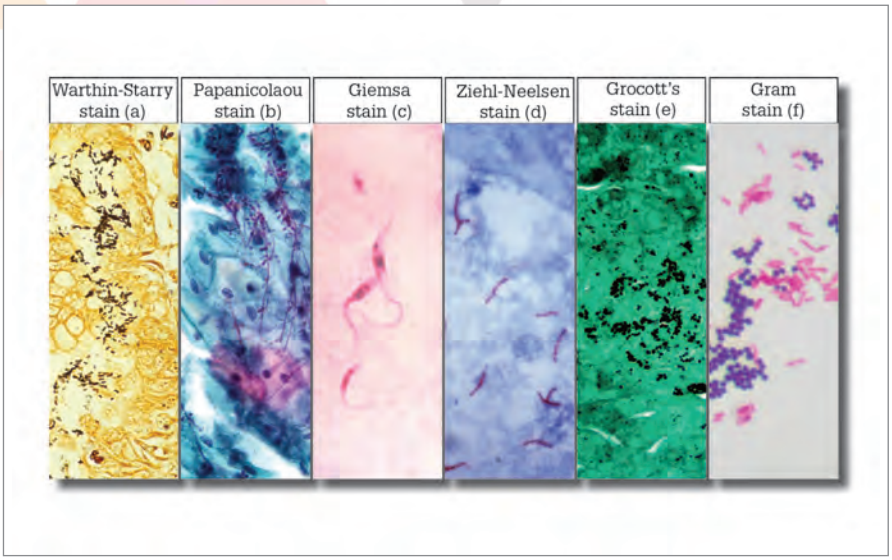

Figure 1. a) *Helicobacter pylori* and spirochetes; b) *Candida* fungal species; c) *Trypanosoma cruzi;* d) *Mycobacterium* species; e) *Histoplasma* fungal species; f) generic bacterial species.

When Hans Christian Gram first discovered that bacterial species could be visually differentiated by the binding of crystal violet to peptidoglycan cell walls, he set in motion a methodology that would revolutionize infectious disease diagnosis and analysis.

Pathogenic species of fungi, protozoa, and bacteria can be thought of as "alien" cells on a background of expected human cells. These pathogens can be discerned through stains that bind infectious agents in a visibly different way to the patient's native tissue – creating a rapid, universal toolkit for diagnosing diseases and matching symptoms to their causative organisms. Even today, color is central to a diagnostician's daily work..

Then and now

In the early days of antibiotics, researchers quickly discovered that beta-lactams, such as penicillin, would only kill bacteria with high cell-wall peptidoglycan content. As a result, the colorful output from a positive Gram stain became a routine indication for treating infections with penicillin.

In modern-day medicine, the scope of infectious and communicable disease has grown exponentially, reciprocated by a wealth of colorful histopathology staining techniques to aid an ever more complex diagnostic process. This means much greater differentiation of increasingly specific infectious diseases can be made during histopathological diagnoses, with each colored stain response acting as a unique identifier on which patient treatment decisions can be made.

Digital pathology – and, in particular, whole-slide imaging (WSI) technology – may overcome caveats to the sharing and analysis of data for a greater breadth of diagnoses in a growing population. Obviously, bright-field light microscopy has some limitations with respect to infectious disease; it cannot resolve most viruses, or even the smallest bacteria, without oil immersion. Nonetheless, some of the world's leading WSI OEM developers and vendors, supported by the brightest optical engineering minds on the planet, are developing technologies that can confidently visualize more species of bacteria, fungi, and protozoa at the limits of bright-field resolution. Although most WSI systems are designed for tissue pathology (and thus limited by resolution and lack of oil immersion), they are increasingly used for infectious disease diagnoses. With further exploration into this novel use of WSI, we can increase the adaptability, ubiquity, and potential of digital pathology devices and analytical software (1). Armed with a catalogue of special stains and resolution limits at the cutting edge of modern WSI optics, we can identify some of the world's most common and clinically relevant infectious diseases (see Figure 1).

The benefits are not limited to human diagnostics – animal and plant science play a significant role in ensuring agricultural supply, developing new antibiotics, and furthering the understanding of disease persistence and antibiotic resistance. The ability of modern WSI scanners to optically resolve these disease agents leads to an interesting new avenue for clinics and researchers – and, like all diagnostic pathways, ensuring the standardization of data reliability, fidelity, and quality across multiple sources forms part of confidence in deployment.

Color cues

Because the colorful special staining of many of these infections is a key differentiator against the host tissue or liquid biopsy background, it is vital to ensure that the digital colors in WSI images remain faithful to their intended targets. Only then can they provide human or artificial intelligence (AI) analysts with valid information on which to base diagnostic decisions. The digitization process, despite offering great advances in data capture rate, storage, and automated analysis, also carries with it some inherent challenges – including accurate color reproduction. Without careful management, this intrinsic caveat of digital imagery could misrepresent the colors used as diagnostic indicators of infectious diseases.

WSI systems often contain basic color handling functions in their image files –

Figure 2. An effective slide-based color calibration slide must cover the whole histopathological spectrum to ensure the optimum coverage of special stains used in infectious disease diagnosis (boxed region), as well as the common H&E range. The inherent errors of all WSI systems ("Before") can be corrected to the real color ("After") through the use of an ICC profile calculated from measurements of slide patches that accurately represent ground truth color data.

from rudimentary, idealized lookup tables to inbuilt physical targets that update color responses on the fly. Unfortunately, many of these functions are limited or biased by their intended applications in clinical oncology and pharmaceutical development and may be in formats that are not representative of the real spectral responses of stained samples. For a WSI system to be universally applicable to infectious disease requires an external, unbiased method that can evaluate the color output from any system. This can identify inherent errors that differ across WSI vendors and provide a mechanism for not only standardizing the color output of these systems, but also ensuring that onward analysis is based on the real color in the original stained sample.

One solution is to apply device-specific International Color Consortium (ICC)-standardized color profiles to all WSI-generated images using real-world spectral data found in stained infectious disease samples. This can be achieved by using a slide with histologically stained patches that precisely mimic real-world colors – for histopathology, common stained tissues; for infectious disease analysis, special stains to identify pathogens. By measuring the spectral absorption of commonly used stains on a tissue-mimicking slide and comparing those measurements with an image of the same slide as scanned by the pathologist, the interpreted error (the scanner's deviation from a standard truth) can be calculated. ICC profile metadata is then generated and used to correct the pre- or post-imaging output so that the digital image contains the ground truth colors.

The color is therefore not only closer to truth, but also unified across all scanners. For human analysis, this means that diagnosis is based upon the same data as in the real sample. In a future increasingly moving toward big data and AI software, applying calibration technology on a regular basis ensures that decisions are based on uniform input – and that all images, regardless of source, remain digitally unaltered and are quality-controlled to be ground truth color as standard. The power that such technology represents can be seen in the ability to correct large errors and high variation across many WSI systems in the spectral response ranges commonly used in infectious disease staining (see Figure 2).

WSI systems must be at peak reliability and data quality to image and detect infectious agents at the current extremes of the technology's reach. For that to happen, we must have control over potential imaging artifacts, such as color misrepresentation, that could skew the sensitive analysis. As for the motivations for general clinical WSI color calibration, the 2016 FDA guidance suggests, "The WSI system should be tested with a target slide. The target slide should contain a set of measurable and representative color patches, which should have similar spectral characteristics to stained tissue" to achieve this level of confidence, fidelity, and validity (2). With big pharma equally concerned with achieving peak levels of GLP acceptability for drug pipelines and software analysis vendors looking to provide solutions that encapsulate and facilitate this drive for standardization and QA, there is a reason for appropriate image data color management in every sector of the WSI market.

The market for WSI systems and data analysis is growing rapidly – which means vendors are under more pressure than ever to be both competitive and in line with emerging accreditation. The field of infectious disease diagnosis is rich with successful technologies – RT-PCR, next-generation sequencing, an array of molecular tests for liquid biopsies – that WSI must match or exceed in throughput and fidelity. The boundaries of imaging speed and quality are likely to be pushed even further, increasing the range of infectious disease detection and therefore increasing the need for stringent data regulation in more clinical applications. Meeting regulatory guidelines is paramount to success, and slide-based color calibration technology is well-positioned to not only provide accuracy and reliability for the current state of play, but also future-proof WSI technologies for use in infectious disease diagnostics.

Richard Salmon is Product Manager for Life Sciences at FFEI, Hemel Hempstead, UK.

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Developing a Robust, Automated, and Streamlined Clinical NGS Workflow for Hematological Malignancies

Next-generation sequencing (NGS) has transformed the field of oncology. Early successes in identifying and targeting oncogenic drivers of solid tumors have set the foundation for genomics-guided precision medicine; but, for hematological malignancies, the path to precision medicine is a lot more complex.

In this application note, we discuss the importance of streamlined clinical NGS workflows within the hematologic-oncology space. To effectively characterize the clinical heterogeneity of myeloid malignancies, routine diagnostic labs must pair highly sensitive, multi-gene assays with indication-specific bioinformatic pipelines that provide up-to-date information on disease classification, prognostication, treatment selection, and monitoring.

Download the application note to learn how to develop a robust, automated, and streamline NGS analysis pipeline for the interpretation and reporting of genomic alterations associated with hematological malignancies.

https://go.qiagen.com/HemeOncNGSAppNote

"There are nearly 175,000 patients newly diagnosed each year who may benefit from diagnostic and prognostic insights elucidated by clinical NGS testing."

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What's in a Name?

How bacteria are named and classified

By Aharon Oren

Many of you will have diagnosed infectious diseases before – perhaps caused by well-known pathogens like *Staphylococcus aureus* or less common ones like *Gemella morbillorum*. But have you ever wondered how these bacteria obtain their names? The process is more organized than you may think…

Naming and classifying

Bacteria have no "official" classification scheme – but they do have formal nomenclature, which is regulated by internationally accepted rules. These rules are fixed in the International Code of Nomenclature of Prokaryotes (1). The International Committee on Systematics of Prokaryotes (ICSP) is responsible for updating and implementing the rules of the Code. Principle 1(4) of the Code states, "Nothing in this Code may be construed to restrict the freedom of taxonomic thought or action." What does that mean? Essentially, that anyone is free to design their own system of classifying bacteria; the Code only deals with the way species, genera, and higher taxa of prokaryotes are named. In recent years, extensive comparative studies of prokaryotic genomes have led to the establishment of the Genome Taxonomy Database (2). The impressive classification system proposed there is widely accepted today; many bacteriologists even consider it "official," even though such a thing does not exist

To obtain standing in the nomenclature, names of new taxa of prokaryotes must be published in the International Journal of Systematic and Evolutionary Microbiology (IJSEM), an official publication of the ICSP. There are two ways of doing this. The first is to publish an original paper describing the new taxon in IJSEM. In addition to the usual scientific peer review, the proposed names are checked by the journal's nomenclature reviewers to ensure that they are formed in accordance with the rules of the Code. However, not everyone may wish to publish in that journal – and, of course, authors are free to publish wherever they wish. The newly proposed names are then considered "effectively published." To obtain the status of "validly published," the authors must then take the second route: a copy of the publication must be sent to the IJSEM editorial office with the request to include the names in the journal's bimonthly Validation List. Such requests must be accompanied by further documentation – in particular, proof that the type strain of the new species and any subspecies are available from at least two culture collections in different countries. The list editors of the journal will check the documents and, if all conditions for valid publication are met, the names will be listed in the next Validation List.

The knowledge of ancient Greek and Latin can play an important role in the process of naming a new prokaryote – but many scientists have only a rudimentary knowledge of classical languages. In fact, the number of microbiologists who have i) the necessary command of Latin and Greek, ii) an interest in nomenclature issues, and iii) most importantly, sufficient time to assist

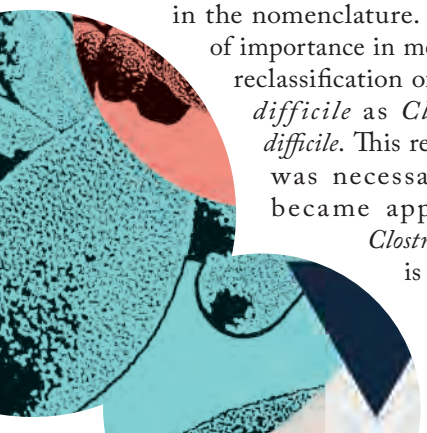
"The knowledge of ancient Greek and Latin can play an important role in the process of naming a new prokaryote."

colleagues worldwide in proposing correctly formed names is very small. Most papers describing new bacterial taxa come from Asian countries in which microbiologists are rarely, if ever, exposed to the classical languages. Often, prospective authors consult me or one of my colleagues in our "nomenclature quality control

team" (3). The editors of some microbiological journals also routinely consult us before they accept taxonomic papers for publication. The final stage is valid publication of the names in the IJSEM following quality control by the nomenclature reviewers and list editors of the journal.

Molecular biology in the lead

Molecular biology is the gold standard of current classification. Since Carl Woese pioneered the use of molecular sequences (notably those of ribosomal RNA molecules) in the late 1970s, molecular data have been used for the classification of bacteria and archaea. The Genome Taxonomy Database is entirely based on sequence data – and, as much as possible, on complete genomes. The results of molecular sequence comparisons do not always agree with the older classification schemes. As a result, many species have been reclassified in new genera as "comb. nov." (combinatio nova, new combination) taxa. In some cases, this has led to considerable confusion. We must remember that the older validly published names retain their standing in the nomenclature. An example of importance in medicine is the reclassification of *Clostridium difficile* as *Clostridioides difficile*. This reclassification was necessary when it became apparent that *Clostridium difficile* is only distantly related to *Clostridium butyricum*, the type species of the genus *Clostridium*.



Renaming medically important bacteria by reassigning them to new genera based on molecular sequence data may cause problems for the medical profession – especially for those involved in diagnosis, classification, and treatment selection. In addition to the case of *Clostridium* versus *Clostridioides*, the genus *Mycobacterium* was recently split into five genera, including the newly proposed *Mycolicibacterium*, *Mycolicibacter*, *Mycolicibacillus*, and *Mycobacteroides*. Some members of the genus *Mycoplasma* were reclassified into the new genera *Malacoplasma*, *Mesomycoplasma*, and *Metamycoplasma*. These names and the new combinations were validly published, but that does not prevent anyone from continuing to use the old names.

"Molecular biology is the gold standard of current classification. [...] The Genome Taxonomy Database is entirely based on sequence data – and, as much as possible, on complete genomes."

Rule 56a of the International Code of Nomenclature of Prokaryotes allows experts to propose the rejection of names "whose application is likely to lead to accidents endangering health or life or both or of serious economic consequences." Only the Judicial Commission of the ICSP can place names on the list of rejected names.

As a service to the medical community, Diagnostic Microbiology & Infectious Diseases periodically publishes a paper entitled, "Proposed Nomenclature or Classification Changes for Bacteria of Medical Importance – Taxonomic Update." The fifth such update, which is now in press, covers the period from 2018 to 2020 and lists 32 names. That may not seem like a lot – but novel pathogens are even rarer. The number of validly published names of newly described human pathogens annually in recent years is in the single digits. Unfortunately, many more names of new pathogenic bacteria are effectively published in the literature, but never submitted to the IJSEM for validation.

Aharon Oren is Professor of Microbial Ecology at the Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Israel. He is also list editor, nomenclature reviewer, and past editor-in-chief of the International Journal of Systematic and Evolutionary Microbiology and past chair of the International Committee on Systematics of Prokaryotes.

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Spotlight on... Technology

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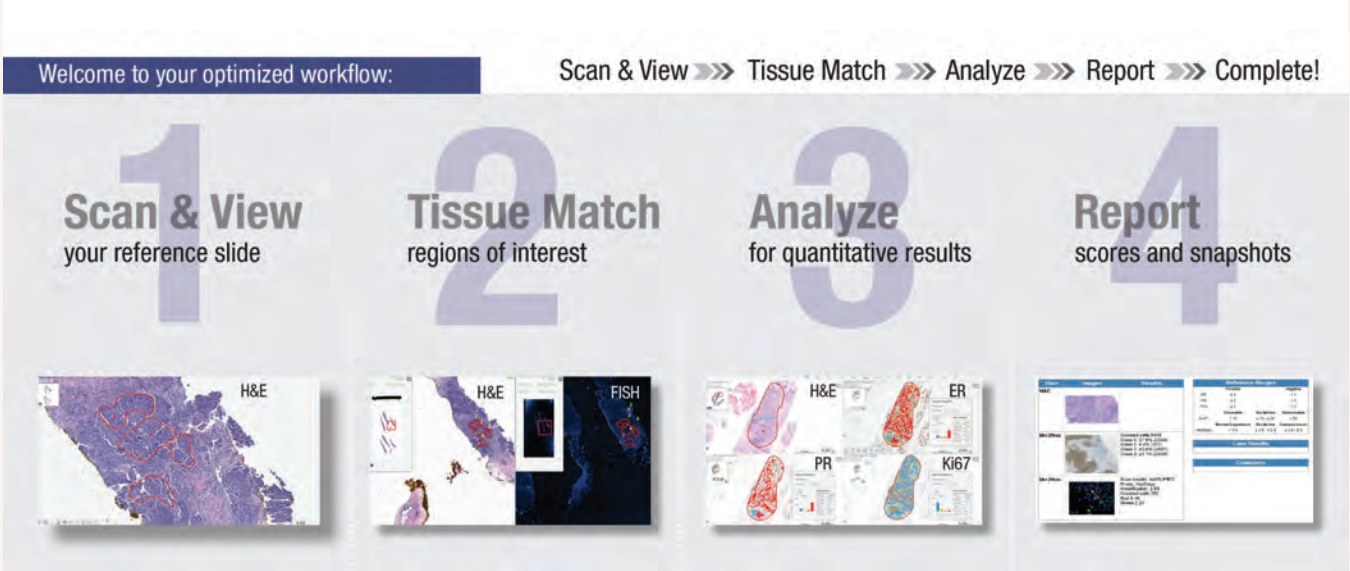

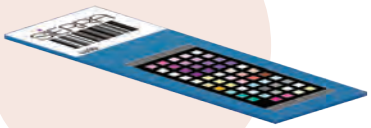

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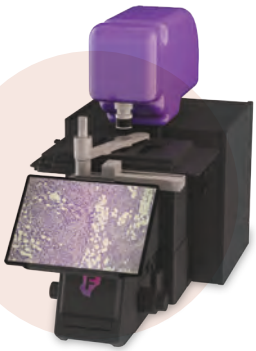

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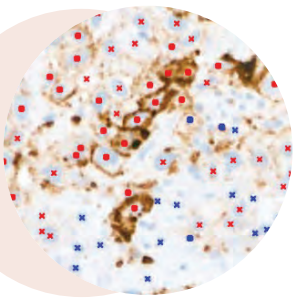

MindPeak Showcases Second Product for PD-L1 After Successful Launch of BreastIHC

After the successful product launch of MindPeak's BreastIHC, our second pilot product for PD-L1 stained cell quantification is coming. Our goal is to relieve pathologists from the burden of PD-L1 scoring in clinical routine and research.
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The View from India

Sitting Down With… Harsh Mohan,
Senior Consultant Pathologist at Oncquest Laboratories, Paras Hospitals, Panchkula, Haryana, India

You have authored eight editions of the Textbook of Pathology. How did this book come about?

In India, until the late 1980s, the only teaching tools I had were a blackboard and chalk. The words spoken by the teacher were considered sacrosanct and the students took notes based on each lecture. The caveat was that students had to consider the teachers "good" – and I met their criteria. In fact, I eventually found out that each upcoming batch of students would photocopy notes of the topics I had taught in previous years and use them to study ahead.

No doubt there were internationally renowned pathology textbooks then, too, but students wanted the material in a format that was easy to understand and reproduce in their exams. I realized that I needed to address this gap systematically; after all, between a fallible teacher lecturing and fallible students taking notes, there were guaranteed to be some transcription errors in the photocopied material that was passed from hand to hand! That is how my long and rewarding journey toward textbook authorship began. The first edition of my Textbook of Pathology was released in 1992. In subsequent years, there have been more pathology books along similar lines written especially for dental, physiotherapy, and paramedical courses. These books have certainly brought me closer to their users all around the world!

Tell us about medical education in India…

India has 536 medical colleges – more than any other country in the world – and admits approximately 80,000 students annually to its graduate program in medicine (the "MBBS"). Many colleges also offer postgraduate courses in various specialties. Indian medical colleges are equally divided between the public and private sectors, but all are under the regulatory control of the Medical Council of India, which supervises them for uniformity of educational standards and for recognition of medical degree qualifications.

At the end of the four-and-a-half-year academic MBBS program, candidates complete 12 months of rotating internships before enrolling as medical practitioners. Those aspiring to pursue postgraduate studies take an additional, highly competitive exam called the National Eligibility cum Entrance Test for Post Graduate courses (PG-NEET). The applicant's score and rank determines their options for postgraduate specialty studies, so most applicants either spend their internship year preparing or take a gap year solely to prepare for the PG-NEET.

Many Indian doctors pursue professional careers in other countries. It is difficult to say exactly how many doctors from India are currently settled elsewhere, but I would estimate that about one in 10 Indian doctors migrates to foreign shores every year. In the USA alone, there are about 40,000 Indian doctors – approximately 20 percent of all international doctors and 5 percent of all doctors in the country!

What is pathology education and training like in India?

About 1,500 students per year are admitted to the three-year pathology course. In the past, pathology seats were in high demand and went to applicants with a high PG-NEET rank. Lately, the job market for pathology has shrunk, so the discipline has become less popular. Ultimately, for some applicants, pathology is their chosen life's work; for others, it is a compromise between what they want and what their PG-NEET rank allows. I have noticed one positive demographic shift; increasing numbers of women are entering pathology, to the extent that – in the not-too-distant future – it may be rare to find a male pathologist in India!

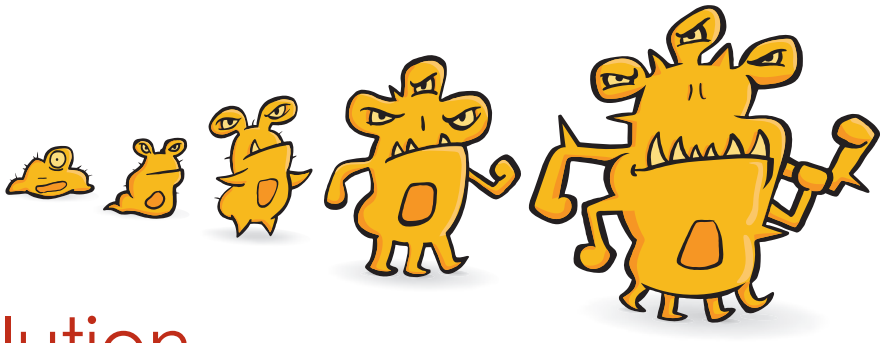
As far as teaching and training in pathology is concerned, there is wide variation between institutions across India. However, training includes rotations through various subspecialties, such as histopathology, cytology, hematology, clinical pathology, and transfusion medicine. At the end of the program, we expect certain standards of knowledge and skill from all candidates. Many have become pathology "household names" – for instance, Vinay Kumar, famous for his work on Robbins' Pathologic Basis of Disease – and some have even started their own organizations.

Where in pathology training in India is there scope for improvement?

I think we need an oversight committee of experts from around the country to establish uniformity in training activities and ensure that all institutions follow a structured academic program. Another problem is that many institutions lack teaching resources. Some don't have the full spectrum of biopsy materials for training; others lack access to modern aids, such as detailed immunohistochemistry panels, molecular pathology, or electron microscopy. This latter issue may be trickier to resolve, but I believe that more privileged institutions should arrange periodic training programs specifically directed at postgraduates in pathology, providing them access to the resources their own institutions may lack.

How do you envision the future of pathology in India?

I think the Internet has led to globalization of knowledge sharing. The current crop of Indian pathology students have access to vast amounts of learning material. Most students are fully aware that it is more important to learn their subject well than to simply pass an exam – and, for them, books are only one of many sources of learning. With respect to the practice of pathology, there is a greater emphasis on quality control and on reporting in the international dataset format. Indian pathologists are striving for greater uniformity and standardization, and I foresee a bright future for us all.



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