

the Pathologist

In My View

Basic laboratory tests are not so simple in cancer patients

16 – 17

In Practice

Working with automation to meet microbiology needs

30 – 33

NextGen

POCT distinguishes bacterial from viral infection

38 – 40

Profession

An online marketplace for experimental outsourcing

46 – 49

Animal Instincts

Meet the furry assistants who are proving valuable in early cancer diagnosis

20 – 27



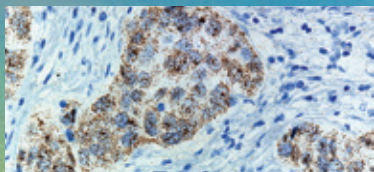
NO ANTIBODY. NO PROBLEM.

Breakthrough multiplex RNA
in situ hybridization for any gene
with RNAscope® technology.

Be Amazed.

Because up to 76% of protein-coding genes have no reliable antibody for immunohistochemistry (IHC), your research often comes to a screeching halt while you wait for a new antibody to be developed. We say forget your antibody problems. Choose RNAscope® technology. With rapid probe design and universal assay workflows for any gene, RNAscope® frees you from the hassles of antibody screening, saving you precious time and effort, while driving publication quality data.

Learn more about RNAscope®
at www.acdbio.com



HER2 expression in human breast cancer
FFPE tissue using RNAscope® 2.0 HD
Reagent Kit-BROWN



Advanced Cell Diagnostics

Image of the Month

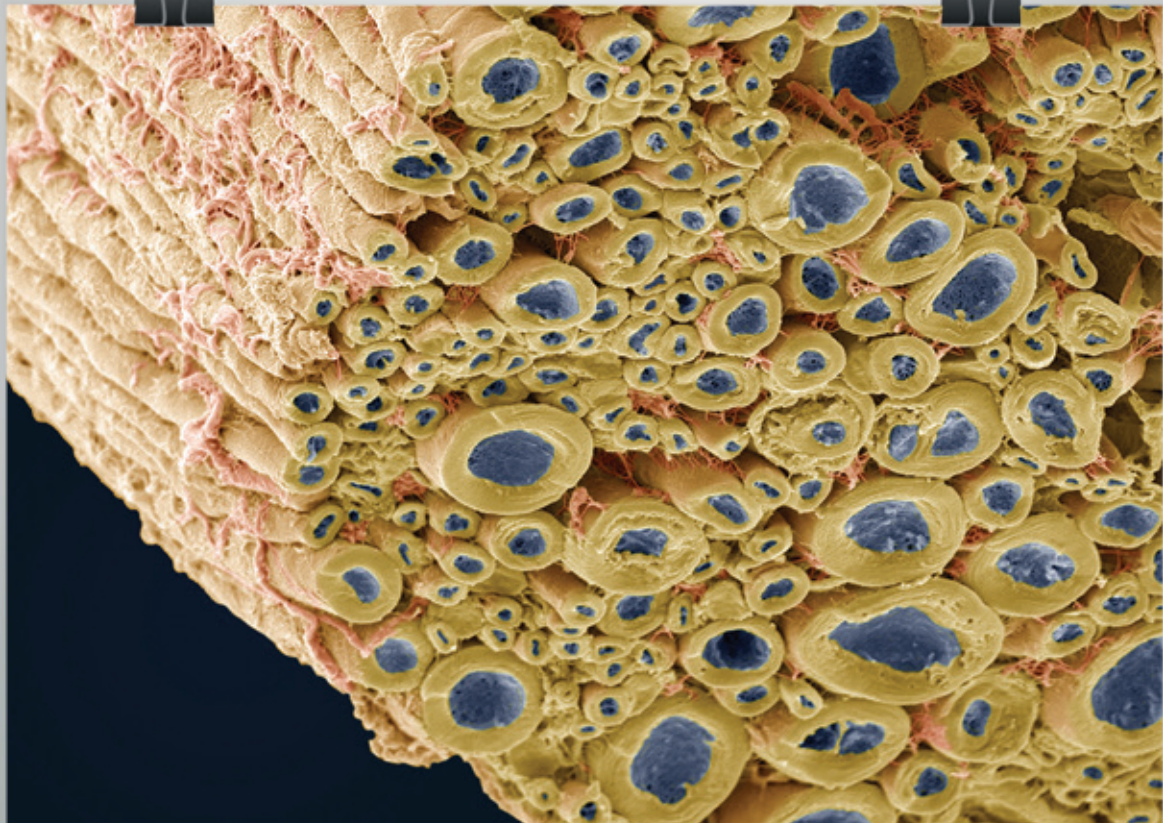


Image of a nerve bundle supplied by scientific photographer Steve Gschmeissner, from Bedford in the UK. The image is a colored scanning electron micrograph of a freeze-fractured section through a bundle of myelinated nerve fibres. Myelin sheaths (yellow) can be seen surrounding the axons (blue). Perineurium (connective tissue, pink) surrounds the nerve bundle while endoneurium divides the individual fibres. Magnification: x1650 when printed 10 centimeters wide. As a side note, Steve needs your help: "I have a number of unidentified micrographs from an excellent histopathology collection of unlabeled slides that I've photographed. If anybody would like to take on the challenge of helping me, please email s.gschmeissner@ntlworld.com."

Do you have an image you'd like to see featured in *The Pathologist*?
Contact fedra.pavlou@texerepublishing.com



10



20

03 Image of The Month

07 **Editorial**
Decision Time,
by Fedra Pavlou

08 Contributors

On The Cover



A dog's nose is a sensitive instrument – and one that may prove an invaluable tool for the noninvasive diagnosis of cancer and other diseases.

Upfront

10 Defining Boundaries

11 Picking Out Parasites

12 Paper (ELISA) Plates

13 Color Critical?

14 Biomarker to Predict Breast Cancer

15 When Is a Cancer Not a Cancer?

In My View

16 **Lakshmi Ramanathan, Elizabeth Wagar and Melissa Pessin** warn of the complexities of interpreting even the most basic laboratory tests in cancer patients.

17 Are you ready for point-of-care testing? **José Luis Bedini** thinks pathologists should rise to the challenge and commit to leading the way in these changing times.

18 **Nancy Hanson** emphasizes the importance of molecular diagnostics in the battle against infection and antibiotic resistance.

Feature

20 **Animal Instincts**
Pet owners have long sworn that their beloved animals could tell when something was wrong. Now, working dogs that can detect the odor of cancer in a urine sample might provide a scientific basis for that statement.

Editor - Fedra Pavlou
fedra.pavlou@texerepublishing.com

Associate Editor - Michael Schubert
michael.schubert@texerepublishing.com

Content Director - Rich Whitworth
rich.whitworth@texerepublishing.com

Publisher - Mark Goodrich
mark.goodrich@texerepublishing.com

Senior Designer - Marc Bird
marc.bird@texerepublishing.com

Designer - Emily Strefford-Johnson
emily.johnson@texerepublishing.com

Digital Content Manager - David Roberts
david.roberts@texerepublishing.com

Mac Operator Web/Print - Peter Bartley
peter.bartley@texerepublishing.com

Tablet Producer - Abygail Bradley
abygail.bradley@texerepublishing.com

Audience Insight Manager - Tracey Nicholls
tracey.nicholls@texerepublishing.com

Traffic and Audience Associate - Lindsey Vickers
lindsey.vickers@texerepublishing.com

Traffic and Audience Associate - Jody Fryett
jody.fryett@texerepublishing.com

Apprentice, Social Media / Analytics - Ben Holah
ben.holah@texerepublishing.com

Events and Office Administrator -
Alice Daniels-Wright
alice.danielswright@texerepublishing.com

Financial Controller - Phil Dale
phil.dale@texerepublishing.com

Chief Executive Officer - Andy Davies
andy.davies@texerepublishing.com

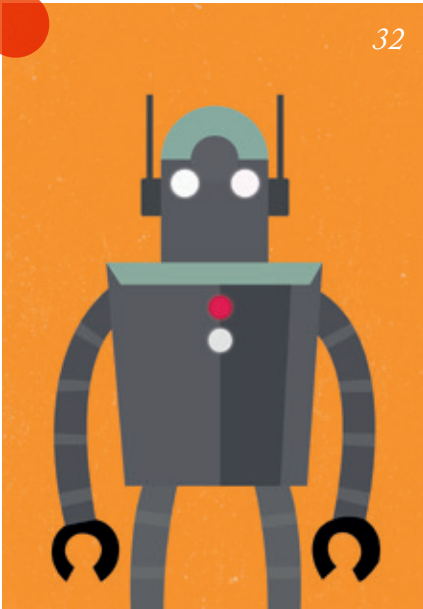
Chief Operating Officer - Tracey Peers
tracey.peers@texerepublishing.com

Change of address:
tracey.nicholls@texerepublishing.com
Tracey Nicholls, The Pathologist,
Texere Publishing Ltd, Haig House, Haig Road,
Knutsford, Cheshire, WA16 8DX, UK

General enquiries:
www.texerepublishing.com
info@texerepublishing.com
+44 (0) 1565 745200
sales@texerepublishing.com

Distribution:
The Pathologist (ISSN 2055-8228),
is published monthly by Texere Publishing
Ltd and is distributed in the USA by UKP
Worldwide, 1637 Stelton Road B2,
Piscataway, NJ 08854.
Periodicals Postage Paid at Piscataway,
NJ and additional mailing offices
POSTMASTER: Send US address changes
to The Pathologist, Texere Publishing Ltd, C/o
1637 Stelton Road B2, Piscataway NJ 08854
Single copy sales £15 (plus postage, cost available
on request tracey.nicholls@texerepublishing.com)
Annual subscription for non-qualified recipients £110

32



In Practice

- 30 **People versus Machines?**
Automation is a necessary next step to expanding and improving microbiology services – but the technology can only work with the support of highly trained laboratory professionals.
- 34 **Disrupting Cytogenetics in the Clinic**
Despite the successes of next generation sequencing, it remains a costly and data-intensive approach. Is mate pair sequencing the solution to affordable, manageable cytogenetics?

Next Gen

- 38 **Pathogens Unveiled**
Distinguishing bacteria from viruses in the lab can take a long time, but not trying can result in poor antibiotic stewardship. A new genetic test may offer a rapid point-of-care alternative.

50



- 42 **Motion Pictures**
MRI+CT fusion allows healthcare providers to study both the morphology and the motion of lung cancers simultaneously, allowing better treatment planning.

Profession

- 46 **Science Searches Simplified**
Elizabeth Iorns, founder of Science Exchange, explains why researchers need an easier way to collaborate and contract out experiments.

Sitting Down With

- 50 **Jennifer Grandis, Professor, Department of Otolaryngology – Head and Neck Surgery, Associate Vice Chancellor – Clinical and Translational Research, University of California, San Francisco, USA.**



Keep your lab running 24/7

Does your water measure up for analysis?

- Photometric reading errors? Your water bath may have particle or bacteria contamination
- Reduced calibration sensitivity? Your diluent may contain ions, organics or bacteria
- Patient sample carryover? You may be washing your probes with impure water

As the Lab Water Specialists with 80 years experience, we know that clinical grade water is essential for meeting your analyzer requirements.

For more useful information download our
Top Tips on Pure Water for the Modern Clinical Laboratory at:
www.elgalabwater.com/pure-water-clinical-lab



The countdown is on and the arguments are ramping up. It's nearly decision day here in the UK (23 June marks the date of the EU referendum) and the pros and cons of a so-called "Brexit" are fiercely being debated. From those in the "stay" camp, Britain is apparently "stronger, safer and better off in Europe than we would be out on our own". Certainly, the notion of strength in numbers is not a new one and it makes perfect sense. But then, doesn't that argument only hold true when the numbers standing together are united and have shared goals? On pondering this, for some reason my mind drifted to military tactics. Perhaps it's my Greek heritage that took those thoughts to the Spartan army, probably the most iconic in military history. They amassed victories against armies far greater in numbers than their own. And in addition to their tactical brilliance, physical strength, bravery and skill, their successes were highly dependent on three key factors: their unwavering focus on shared goals; a mutual respect for one another; and, most importantly of all, complete unity. Can EU member states with differing infrastructures, economies, cultures and goals ever form a unit that is strong enough to emerge victorious against the odds?

Though not on as big a scale, laboratory and pathology services are now facing a similar dilemma – stand together or go it alone? Why? Workloads are increasing. Substantially. Populations are aging, knowledge of the molecular basis of disease is expanding, pressures to instate or expand molecular diagnostic services are rising, the need for digitization and automation is becoming ever more pressing... And with all of this comes the necessity for continuous training and education – in a profession that is already overstretched and time-poor. So, the big question that most laboratories now face is: do we bring all capabilities in-house and retain complete control, or collaborate with complementary service providers for a turnkey solution? This is a really tough question to answer. It may be that your department's financial status will force a decision on you. Either way, what's arguably more difficult than the decision itself, is the pressure to make it work.

I hear many opinions from those who speak passionately of retaining full control of their diagnostics services versus others who believe in the power of partnership. And it's clear that one size does not fit all. But we're here to help. If you have a story to tell or an opinion that you would like to share on this issue, why don't you unite with us and we'll give your story a stage? My contact details are on the masthead.

Fedra Pavlou
Editor



Elizabeth Iorns

After completing a doctoral degree in breast cancer research, Elizabeth was working as an assistant professor at the University of Miami, USA, when she became aware of an unmet research need – simple experimental outsourcing with clearly defined ownership and incentives. In response to that need, she founded Science Exchange, an online marketplace where researchers and service providers can find one another and collaborate with ease. When not working on Science Exchange, Elizabeth is a part-time partner at accelerator program Y Combinator and mentors at IndieBio. Read about Elizabeth and Science Exchange on page 46.



José Luis Bedini

José is Head of the Core Lab, Diagnostic Biomedical Center, Hospital Clínic de Barcelona, Spain, and Professor of Biochemistry at the University of Barcelona's medical school. His main interests are in POCT and laboratory automation and management, in which he has presented more than 100 national and international lectures. He's currently President of the Spanish National Commission for Clinical Chemistry and Laboratory Medicine, and has previously been President of the Spanish Committee of Automation and Analytical Systems and of the Spanish Committee of Laboratory Management.

José urges pathologists to take a proactive approach to implementing POCT and to recognize its strengths and limitations on page 17.



Lakshmi Ramanathan, Elizabeth Wagar and Melissa Pessin

Lakshmi Ramanathan is the Service Chief of the Clinical Chemistry Service, Department of Laboratory Medicine at Memorial Sloan Kettering Cancer Center where she manages a staff of more than 40. Speaking of the impact of cancer on laboratory tests, she says, "It's an incredible field, but the paucity of information makes it very challenging."



Elizabeth Wagar is a Distinguished Professor and Jose M. Trujillo Endowed Chair of the Department of Laboratory Medicine, University of Texas MD Anderson Cancer Center. She has extensive experience in academic medicine and laboratory administration at two major academic medical centers and has been on numerous CAP and ASCP commissions and committees.



Melissa Pessin is the Chair of the Department of Laboratory Medicine at Memorial Sloan-Kettering Cancer Center. Graduating with degrees in electrical engineering and computer science, she was delighted when she found a perfect career fit in laboratory medicine. "It integrates all aspects of my training and offers enough variety that I am never bored. I feel like the luckiest person in the world to have found a job that I love!" she says.

Lakshmi, Elizabeth and Melissa discuss the challenges of conducting even the most basic lab tests for cancer patients on page 16.

See how you can
guide the path
her cancer takes



At the advanced edge of oncology, rapid access to accurate data on disease state is vital. The fully automated Idylla™ system, in combination with the Idylla™ ctBRAF Mutation Assay (liquid biopsy), is the future of diagnosis and monitoring.

With first-time-right results you create the opportunity for disease interception - changing the course of your patients' cancer.

To join the investigation visit www.biocartis.com



The Idylla™ ctBRAF Mutation Assay is available for Research Use Only, not for use in diagnostic procedures. The Idylla™ system is available as a CE-marked IVD. Not for sale in the US and Canada.

AD2006-0316BEN



Upfront

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

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

Email: fedra.pavlou@texerepublishing.com



Defining Boundaries

Third harmonic generation microscopy could allow surgeons to spot and remove tumor tissue in real-time

When a surgeon operates on a brain tumor, it can be medically challenging to tell whether or not they've managed to remove all of the necessary tissue. Correctly identifying the margins of the tumor can be further complicated if there is damage from previous surgery or other anti-cancer therapies, and even the best of eyes will fail to detect a few microscopic cells left behind. That is, of course, where the pathologist comes in. Identifying the presence of remaining tumor tissue is a crucial element of the treatment process, but it's a step that generally takes place once surgery is complete, which is often not ideal.

Clearly, a solution that supports the identification of tumor tissue during surgery would help avoid unnecessary further treatment and distress to the patient, and would save time and presumably costs. That's what researchers from the Vrije Universiteit Amsterdam thought when they devised a near-real-time, label-free method of detecting tumor tissue in the brain (1).

Third harmonic generation microscopy (THG) involves firing photons of a given wavelength into tissue; when three photons simultaneously interact with the tissue, the reaction produces a single photon at one-third the wavelength (and triple the frequency), which is picked up by a detector to generate an image of the tissue (see Figure 1). Because the technique is so clear – allowing visualization of subcellular features – and so fast – ranging from under one second to five minutes, depending on image size and detail – it's possible to apply it during surgery, allowing neurosurgeons to assess tumor boundaries while there's still time to act. “The special

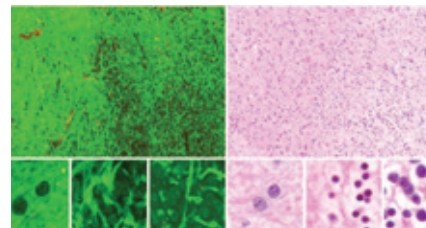


Figure 1. Imaging of a low-grade glioma sample using THG microscopy (left) and conventional hematoxylin and eosin staining (right). Credit: N.V. Kuzmin et al., VU University Amsterdam, The Netherlands.

thing about our images is that we showed they contain so much information,” said principal investigator Marloes Groot (2). “When I showed these images to the pathologists that we work with, they were amazed.” Although THG isn't a new technique, this is the first time it has been used on human brain tumor samples – and the outlook is promising.

What's next for Groot and her colleagues? Now that they've established that THG works on tumor samples, they'd like to construct a tabletop THG device for placement in an operating room, so that it can provide immediate feedback to surgeons during complicated operations. They're also working on a new device to overcome one of THG's current limitations: the fact that its laser pulses can only penetrate about 100 μm into a given tissue. They hope to develop a THG-based bioptic needle to deliver photons below the tissue surface for greater reach, potentially expanding the technique's usefulness. Such a device might even be able to yield diagnostic information prior to or instead of surgery – not just in brain tumors, but for a wide variety of histopathological applications. *MS*

References

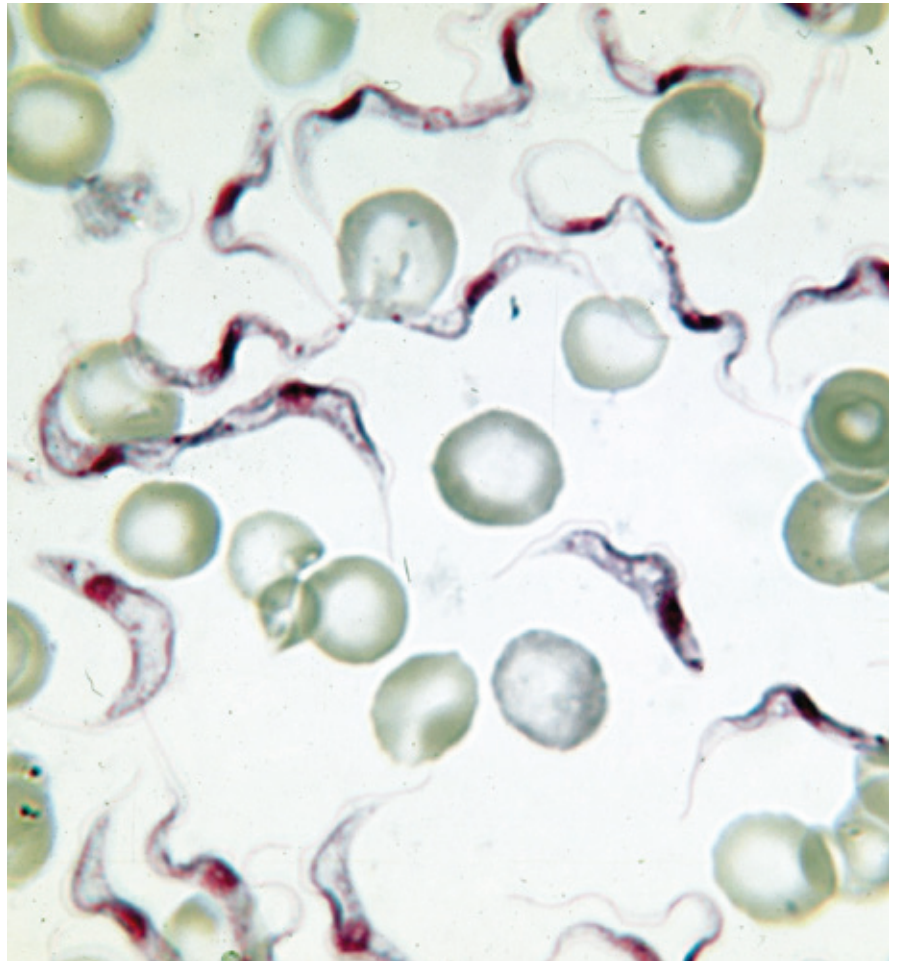
1. NV Kuzmin et al., “Third harmonic generation imaging for fast, label-free pathology of human brain tumors”, *Biomed Opt Express*, 7, 1889–1904 (2016).
2. *MedicalXpress*, “New method allows surgeons to identify brain tumors in real time”, (2016). Available at: <http://bit.ly/1R3BFAe>. Accessed May 16, 2016.

Picking Out Parasites

A new microfluidic device offers quick, affordable trypanosome detection and separation in the field

Even in a fully equipped laboratory, diagnosing tropical diseases isn't always easy. Blood parasites must be detected against a massive background of normal cells – a task to which complex microfluidic tools with pressure regulation, monitoring and microscopy are well-suited. Unfortunately, those tools aren't usually available in the field, where blood parasite diagnosis is most needed but resources can be extremely limited. Jonas Tegenfeldt and his colleagues took on the challenge of finding a solution that works in all environments.

“A number of parasitic blood infections decimate populations in the developing world,” says Tegenfeldt. “Diagnosing these diseases remains problematic.” He and his team had been working on microfluidic cell sorting for many years when it occurred to them that differences in shape and motility would make the parasites amenable to separation from normal blood cells, and this separation could be achieved using a simple device. Tegenfeldt explains, “Three different separation schemes are combined into a single device. First, white blood cells are removed from the blood. Second, the remaining red blood cells and parasites are moved to the side to create an empty stream of plasma, into which the parasites are focused in the third step. Each step requires an optimized design for its particular task, which presented both a fabrication challenge – to accommodate different depths – and a fluidics



Trypanosoma brucei protozoa.

challenge – to align the flow of the fluid so that the three different sections work well together.” The platform they devised is based on deterministic lateral displacement, deflecting particles in different directions based on size (1). It's the size of a microscope slide, contains inexpensive materials, has no moving parts, and requires no power, making it an ideal solution for underprivileged areas.

It's currently quite difficult to diagnose diseases like African trypanosomiasis because cases must be confirmed by positive identification of parasites in the blood – and they can be hard to find. “Some infections are at extremely low

level,” Tegenfeldt warns. “There may be as few as 10 of these cells in one milliliter of blood, so it's important to take sufficient volumes and to pre-enrich the parasites to get successful separations.” He and his group are currently working toward linking their device with a pre-enrichment step, in the hope that they can make diagnosis easier and enable faster treatment for patients with parasitic disease. *MS*

Reference

1. SH Holm et al., “Simplifying microfluidic separation devices towards field-detection of blood parasites”, *Anal Methods*, 8, 3291–3300 (2016).

Paper (ELISA) Plates

A new type of platform for HIV/HCV co-infections could offer an affordable, portable and easy to use diagnostic solution for resource-poor settings

Human immunodeficiency virus (HIV) is one of the leading causes of death worldwide, especially in rural countries, and it's estimated that up to one-third of HIV-positive individuals are co-infected with hepatitis C (HCV), which unsurprisingly affects their care needs and survival rates. Because existing tests either require fully equipped clinical laboratories or lack accuracy, it's difficult to identify these patients in rural or resource-limited settings. Xinyu Liu, part of a group developing a portable, low-cost HIV/HCV testing platform, is tackling the challenge.

What inspired you to develop a low-cost platform for HIV and HCV testing?

This project was initiated through a Star in Global Health Award granted by Grand Challenge Canada. Our goal was to develop a low-cost diagnostic platform for use in African countries where sexually transmitted infections (STIs) like HIV and HCV are major life-threatening diseases. Although rapid point-of-care tests (POCT) for HIV/HCV have been used in the past, we still very much need new diagnostic platforms that are affordable, but don't compromise on high accuracy, sensitivity and throughput.

The ideal setting for use of the platform is in small clinics in remote or resource-poor settings with limited access to laboratory services, such as clinics in developing countries like Kenya, or community clinics in rural and northern Canada. It's also useful for the care

of elderly or disabled patients whose conditions need to be monitored at home.

How does your paper-based test work?

The platform includes a paper device with eight disposable electrochemical biosensors and a custom-made, low-cost, handheld potentiostat (an electrochemical reader). It allows users to carry out eight simultaneous ELISAs – four for HIV and four for HCV antibodies – providing a higher throughput than existing HIV/HCV POCTs. To run a test, you simply insert the paper device into the potentiostat, add microliter drops of serum sample and reagents to the eight biosensors, and trigger the electrochemical measurement by pressing a button on the potentiostat. The results can be displayed on the potentiostat's LCD screen, or transmitted to a smartphone, computer or remote site for telediagnosis or healthcare data collection (Figure 1).

What sets the new device apart from previous testing methods?

Our technology puts ELISA, one of the most commonly performed clinical tests, on a highly portable and inexpensive platform. It requires no laboratory infrastructure, very low-level operator skills, completes eight tests in parallel within 20 minutes, and provides quantitative results with comparable accuracy, sensitivity and specificity to clinical analyzers. It can also telecommunicate, making it compatible with existing e-health systems. The paper-

based device only takes three microliters of serum sample per test, so the blood from a fingerprick should be enough to perform the diagnostics.

Our platform is still a laboratory prototype, though, and requires further development. It can't handle whole blood samples at this point, so blood drawing and separation are still needed. We're working to incorporate a membrane into the device for on-chip plasma separation. This will allow the test to be run directly from a fingerpick sample. Also, although our laboratory calibration experiments have demonstrated satisfactory performance, we still need to conduct a systematic evaluation of our device against gold-standard ELISA using patient blood samples to further verify its clinical performance.

How might it change the day-to-day work of those involved in HIV and HCV testing?

The platform was designed not to replace conventional molecular diagnostic tests routinely performed in well-equipped laboratories, but to provide a low-cost and easy-to-operate alternative for use elsewhere. It allows rapid and accurate HIV/HCV testing by less skilled operators at the point of care, and could facilitate the day-to-day work of pathologists and laboratory professionals who need to perform HIV/HCV tests outside laboratory environments.

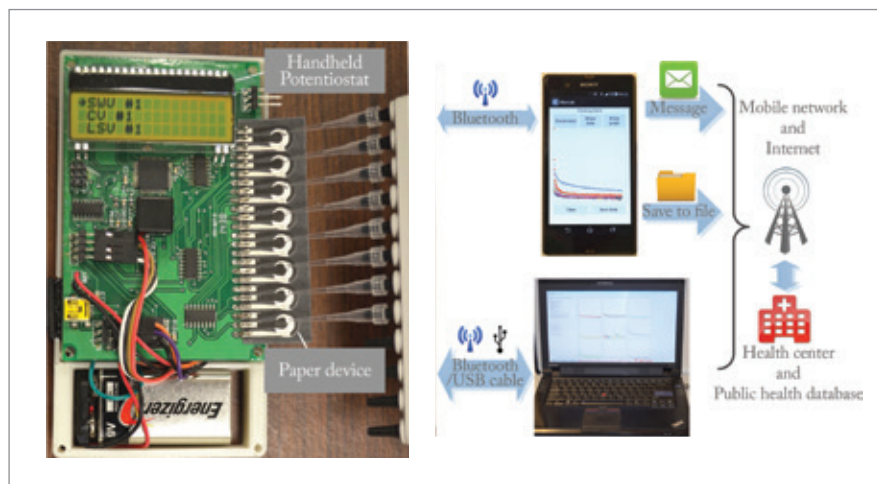


Figure 1. The path of a sample through the portable, paper-based HIV/HCV test developed by Zhao, Liu and their colleagues.

Credit: C. Zhao/McGill University.

The technology isn't only applicable to HIV/HCV, but to many molecular diagnostic tests. ELISAs are widely used in clinical laboratories to detect various antigen and antibody disease markers, so our test could be adapted to diagnose any disease using such a biomarker. For instance, we're currently pursuing testing for cardiovascular diseases and cervical cancer. In the future, the platform could also be used for nucleic acid tests, also widely used in diagnostics.

But first, we need to ready the platform for clinical use by performing real patient sample testing in Canada and Kenya, and by

further developing the engineering aspects of the platform for technology transfer. We expect to achieve initial adoption of a commercial version of the device in Kenyan clinics within five years, but its adoption in western countries – where market barriers and regulatory processes are more complex – will take longer.

Reference

1. C Zhao, X Liu, "A portable paper-based microfluidic platform for multiplexed electrochemical detection of human immunodeficiency virus and hepatitis C virus antibodies in serum", *Biomicrofluidics*, 10, 024119 (2016). PMID: 27158287.

Color Critical?

Researchers challenge the importance of color in dermoscopic diagnoses

When evaluating skin lesions, it's clear that both color and structure matter. The common acronym for diagnosing malignant melanoma – ABCDE (asymmetry, borders, colors, diameter, enlarging) – includes both, and dermatologists and pathologists involved in diagnosis are trained to recognize abnormalities in either. But just how much weight each factor carries has been unknown until recently, when the results of a study conducted at the 2014 Memorial Sloan Kettering Cancer Center dermoscopy course were published in *JAMA Dermatology* (1).

Researchers sought to determine the effect of color images on diagnostic accuracy by showing participants a sample of 40 various skin lesions. Of those, half were shown only once (10 in color and 10 grayscale); the other half were shown twice, once each in color and grayscale. The 158 participants in the study – with an average of six years' experience evaluating skin lesions and two using dermoscopy – were asked to both

provide a diagnosis and rate their confidence level in their conclusion. The results were surprising. When shown unpaired images, univariate analysis suggested that participants were less likely to diagnose color images correctly than grayscale. Multivariate analysis found no association between color images and correct diagnoses. A stratified analysis of paired images further revealed that participants were more likely to diagnose dermatofibroma correctly in grayscale, but squamous cell carcinoma and hemangioma in color.

Overall, the authors concluded that a lesion's morphological characteristics provide more powerful diagnostic clues than its color. They also proposed teaching dermoscopy to novices using grayscale images in order to emphasize the value of looking at the structures and patterns of lesions. This doesn't mean that color isn't an important aspect of the diagnostic evaluation – but next time you're facing a tricky diagnosis, you may want to look at the image in black and white... MS

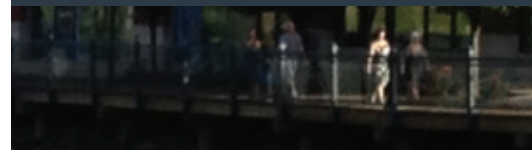
Reference

1. S Bajaj et al., "The role of color and morphologic characteristics in dermoscopic diagnosis", *JAMA Dermatol*, [Epub ahead of print] (2016). doi:10.1001/jama.2016.11111. PMID: 27007917.



Gaithersburg Marriott Hotel,
Gaithersburg, MD

CMC STRATEGY FORUM
ADVANCING BIOPHARMACEUTICAL DEVELOPMENT



July 18-19, 2016

Emerging Strategies in Drug Product Comparability and Process Validation

Forum Co-chairs:

Howard Anderson, CDER, FDA
Yves Aubin, Health Canada
Zahra Shahrokh, STC Biologics
Andrew Weiskopf, Biogen

July 20-21, 2016

Change Happens: Technical and Regulatory Considerations for Pharmaceutical Product Lifecycle Management

Forum Co-chairs:

Julia Edwards, Biogen
Joseph Kuitza, MedImmune, A member of the AstraZeneca Group
Emanuela Lacana, CDER, FDA
Ingrid Markovic, CBER, FDA



CASSS
SHARING SCIENCE SOLUTIONS

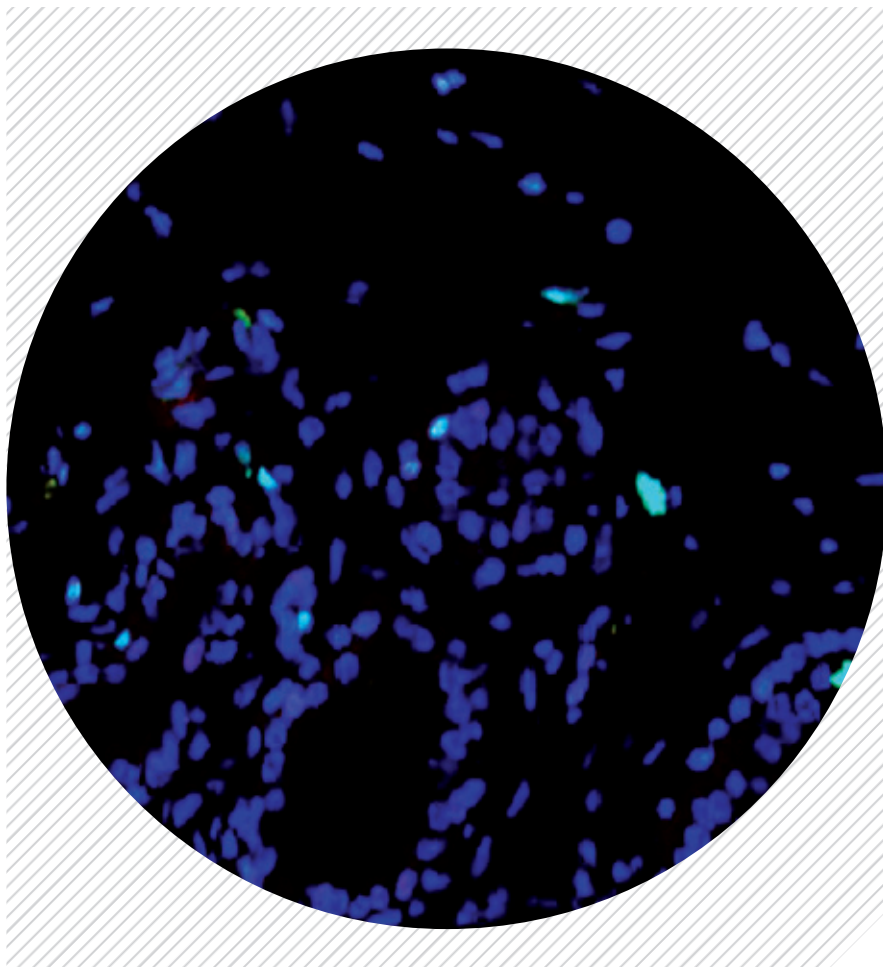
Please browse the Forum Web site
for program updates: www.casss.org

Biomarker to Predict Breast Cancer

Ki67 in healthy breast tissue may indicate increased risk of developing the disease

The need for preventative measures against cancer is clear – if we can detect it early, or better yet, predict which patients are likely to fall victim, we can increase the odds of survival. Although many good screening programs exist for breast cancer, the accuracy of diagnosis still cannot be guaranteed. A marker that can predict the risk of developing the disease in the first place should help drive down the rates of late or missed diagnoses and improve patients' chances of survival. Researchers Kornelia Polyak (Harvard Stem Cell Institute), Rulla Tamimi (Brigham and Women's Hospital) and their colleagues think they may have identified just such a marker.

Ki67 is a nuclear protein associated with proliferating cells. Although it is currently tested in known tumors to assist in treatment decision-making, its presence and role in healthy tissue haven't been investigated. Polyak and Tamimi examined biopsies from 302 patients diagnosed with benign breast disease, 69 of whom eventually developed breast cancer and 233 of whom did not (1). They found that, even in non-cancerous tissue, the women who developed cancer had significantly higher levels of Ki67 in the mammary epithelium, where most breast tumors originate. In fact, women with high levels of Ki67 and low levels of either p27 (a tumor suppressor) or estrogen receptors exhibited a five-fold higher risk of breast cancer than women with low Ki67 levels. The



Healthy breast tissue, showing nuclear staining of individual cells (blue). Few cells expressing Ki67 (green) and p27 (red) are present.

researchers believe that testing the biopsies of at-risk women, such as those with known *BRCA1* or *BRCA2* mutations, could identify those at greatest risk of developing breast cancer. Not only could this inform monitoring and treatment strategies going forward, but it could also help women avoid unnecessary additional screening – minimizing discomfort and radiation exposure for patients and reducing costs for healthcare systems.

The question is, could Ki67 testing for this particular purpose move to the clinic in the near future? Polyak doesn't think it would be difficult once the

researchers have replicated their results; after all, the test already exists for tumor profiling and could easily be applied to healthy tissue from high-risk patients. Although not yet ready for clinical use, it may not be long before breast cancer risk assessments are conducted with the use of a quantitative biomarker. *MS*

Reference

1. SJ Hub et al., "The proliferative activity of mammary epithelial cells in normal tissue predicts breast cancer risk in premenopausal women", *Cancer Res*, [Epub ahead of print] (2016). PMID: 26941287.

Credit: Sung Jm Hub

When Is a Cancer Not a Cancer?

New diagnostic criteria for thyroid neoplasms prevents aggressive treatment of indolent tumors

Thyroid cancer incidence is on the rise. In the last two decades, the number of diagnoses per capita has more than doubled (1) – in part due to early detection. But unlike in most cancers, where early detection is key to treatment success, this may actually be having a negative impact on thyroid cancer patients. Why? Because many of the tumors detected may, despite their abnormal cellular appearance, be non-progressing. And if the tumor isn't growing or harming the patient, then the treatment could, in fact, cause more damage than the disease.

But now, an international panel of experts has taken the matter in hand. A group of 25 pathologists, four clinicians, one statistician and one patient have collaborated to reclassify a particular type of indolent thyroid tumor as a non-cancer (2), changing its recommended treatment path. The tumor is known as encapsulated follicular variant of papillary thyroid carcinoma, or EFVPTC, and the panel determined that not all types of EFVPTC are created equal. "The previous classification system classified all EFVPTC, with invasion and without invasion, as cancers," explains Yuri Nikiforov, senior investigator on the project. "However, a growing body of evidence suggested that noninvasive EFVPTC are highly indolent tumors with very little chance of hurting the patient. Nevertheless, these patients were treated the same as invasive cancers. Patients were left with the psychological and medical impact of being diagnosed with cancer, including unnecessary removal of the thyroid, lifelong follow-up appointments and medications, and the financial and psychological burdens."

To reach their conclusions, Nikiforov and his co-panelists independently reviewed 268 EFVPTC cases, including patients' follow-up care for up to 26 years. In noninvasive tumors, patients experienced no recurrence, metastasis or other manifestations of disease at any point. Based on the data, the panel reclassified noninvasive EFVPTC as noninvasive follicular thyroid neoplasm with papillary-like nuclear features, or NIFTP, discarding the word "cancer" altogether. Nikiforov estimates that these types of tumors make up 10 to 20 percent of all thyroid cancer diagnoses and recommends, "Pathologists need to get familiar with the diagnostic criteria for NIFTP and indicate in their reports the risk of recurrence (<1% in 10 years). Importantly, the entire capsule of the tumor must be examined microscopically to exclude capsular and vascular invasion." *MS*

Characteristics of NIFTP

Encapsulation or demarcation

Follicular growth pattern with <1% papillae and no psammoma bodies

Presence of nuclear features of papillary thyroid cancer (nuclear score 2–3)

Exclusion Criteria

>30% solid, trabecular or insular growth pattern

No capsular or vascular invasion

No tumor necrosis

No high mitotic activity

References

1. Cancer Research UK, "Thyroid cancer incidence trends over time" (2016). Available at: <http://bit.ly/1Ws1ZvQ>. Accessed May 12, 2016.
2. YE Nikiforov et al., "Nomenclature revision for encapsulated follicular variant of papillary thyroid carcinoma: a paradigm shift to reduce overtreatment of indolent tumors", *JAMA Oncol*, [Epub ahead of print] (2016). PMID: 27078145.

Equipment for Histo-Pathology Labs

COLOR UP YOUR LAB



In My View

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

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

Contact the editor at fedra.pavlou@texerepublishing.com

The Limitations of Cancer Tests

Interpreting routine laboratory tests in oncology applications can be challenging



By Lakshmi Ramanathan, Service Chief of the Clinical Chemistry Service, Department of Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, Elizabeth Wagar, Distinguished Professor and Jose M. Trujillo Endowed Chair of the Department of Laboratory Medicine, University of Texas MD Anderson Cancer Center, Houston, Texas, and Melissa Pessin, Chair of the Department of Laboratory Medicine at Memorial Sloan-Kettering Cancer Center, New York, USA.

Cancer patients present unique challenges for even the most basic laboratory testing. The frequency of these challenges is increasing in line with the growing incidence and prevalence of cancer in our aging populations. And the numbers are telling: in 2015, the US cancer incidence was ~1,658,370, with a mortality of 589,430, while predictions for 2030 suggest a global incidence of 21.7 million and a mortality of 13 million (1).

Health workers are dependent upon accurate laboratory results for monitoring their patients' progress and for making timely and informed decisions on their treatment and care. However, it is important to recognize that many laboratory tests are affected in unexpected ways by the patient's disease or medication; therefore, it is critical

to take account of the entire clinical picture when interpreting test results. In cancer patients, for example, normal cellular mechanisms involved in inflammation, wound healing, and hemostasis are hijacked by tumor cells to support proliferation and metastasis. So markers of the activation of these processes become useless in cancer patients, particularly as the disease advances. Examples include acute phase reactants such as C-reactive protein (CRP), haptoglobin, and ferritin; the coagulation cascade markers, D-dimer and fibrinogen; and the inflammation markers procalcitonin and lactic acid. All of these can become markedly elevated in cancer, often to much higher levels than are normally seen in the usual activation of these pathways in other diseases.

Furthermore, cancer patients may exhibit unique result outliers for nearly every analyte in the standard comprehensive metabolic panel. For example, although potassium levels may be truly elevated in cancer patients, it is more common to find that results are falsely elevated by pre-analytical errors such as hemolysis. Pseudohyperkalemia can also occur when high numbers of fragile cells (for example, leukemia cells) are centrifuged or otherwise manipulated. And other complications may arise due to the production of discordant low or high potassium levels – known as reverse pseudo-hyperkalemia – associated with some heparin collections.

Different cancers may bring different challenges. Patients suffering from multiple myeloma are particularly problematic due to elevated levels of protein in their serum. Interference of serum protein with test methodologies may give spurious results, such as pseudohyperbilirubinemia, pseudohyponatremia, pseudochloridemia, pseudohypercalcemia, falsely low albumin, pseudohypolipidemia, pseudohyperphosphatemia and pseudohypophosphatemia. Corrections or alternate methodologies are therefore recommended in such cases.

Naturally, tumor markers are among the analytes normally monitored in cancer patients. An ideal tumor marker test should have the following five attributes: high positive and negative predictive values; inexpensiveness and simplicity; clearly defined reference levels; patient acceptability; and validation from a large prospective trial. Unfortunately, no such ideal tumor marker exists! And with the current generation of tumor marker tests, tremendous variability is seen in the results generated by different products and methodologies. Efforts are underway to minimize variability by standardizing these tests, but this is an ongoing process. In addition, methodologies need to be checked for uncommon but potentially important interferences due to cross-reactivity and heterophile antibodies. Inter-laboratory variation may also be problematic, and where possible should be eliminated by using the same laboratory and methodology for tumor marker tests.

Also, we need to be aware of non-

routine cancer markers, particularly where a poor appreciation of their link to cancer may contribute to a misdiagnosis or delay in treatment. The human chorionic gonadotropin (hCG) marker used for pregnancy testing is a perfect illustration of this problem. In addition to its various degradation products, the hCG molecule has five different forms: intact hCG and hyperglycosylated hCG molecules (consisting of an alpha and a beta chain), produced by the placenta during pregnancy; sulfated hCG, produced during the menstrual cycle by the pituitary gland; and hCG beta and hyperglycosylated hCG beta, produced by advanced malignancies. The different hCG forms are excreted in urine with varying efficiency, such that the isolated beta chain is most abundant. Furthermore, different pregnancy testing methods detect the different forms with varying efficiency (which we should expect, because the tests were only developed for comparing pregnant with non-pregnant healthy

females). Given that up to 48 percent of cancers have detectable urine hCG beta, a number of non-pregnant cancer patients will test positive on a routine pre-surgical urine pregnancy screen. This often results in significant delay of surgery for these patients.

Finally, new treatments for cancer, such as immunotherapies, can have unexpected effects on laboratory testing. For example, we have seen an experimental antibody therapy for multiple myeloma, aimed at the CD38 antigen, cause a positive pan-agglutinin-type result in the blood bank antibody screen.

In summary, the best advice for laboratory staff is to work closely with their oncology colleagues when unexpected laboratory results occur in cancer patients, as it is very possible that the cancer or the treatment is playing a significant role.

Reference

1. American Cancer Society, 2015.

Rising to the POCT Challenge

Labs must take a proactive approach to implementing point-of-care testing and recognize their limitations and when use is and isn't appropriate



By José Luis Bedini, Head of the Core Lab, Diagnostic Biomedical Center, Hospital Clínic de Barcelona, Spain.

POCT has applications in diverse settings, including emergency departments, general practice (physician office laboratories), community testing (pharmacies) and patients' homes (self-monitoring, e.g. for diabetes and coagulation). Professionally, it is one of my main areas of interest; but it also causes me much concern. Clearly, demand for new POCT devices is being driven by an ever-expanding range of clinically relevant analytes; at the same time, innovative device development is being enabled by rapid advances in technology, informatics, and automation. This is resulting in the design and manufacture of a broader range of better

POCT instruments that deliver higher quality performance. From my point of view, however, technology alone is not sufficient to benefit either the individual patient or the healthcare process as a whole.

The first thing to consider is the true reason behind any proposal to introduce POCT. Yes, POCT provides fast results and consequently permits quicker healthcare decisions, but that does not make it the answer to disorganized workflows or inefficient laboratories, which are not uncommon in hospital settings. In fact, turnaround time can be significantly improved, without recourse to POCT, simply by a careful review of all steps within the testing process from the pre-analytical to the post-analytical phase. In many circumstances, then, adoption

of POCT is unnecessary, and I would recommend that POCT is introduced only when conventional laboratory solutions prove to be inadequate for meeting specific healthcare demands.

Another consideration is the reception of POCT by the various stakeholders in the clinical testing process. Often, workers outside of the laboratory won't readily assume additional tasks they don't consider part of their regular duties. Consequently – at least during the initial implementation of POCT – they may reject the introduction of “laboratory devices” on hospital wards. Clearly, this can be challenging. However, by creating multidisciplinary groups – to include nurses, clinicians, technicians and laboratory staff – at the start of the project, we can give everyone the chance to get involved in selecting the appropriate device and in planning the implementation process. Such stakeholder buy-in significantly facilitates the introduction of new technology.

Training the users of new devices is another important aspect of POCT uptake, and is also one of the laboratory's responsibilities. POCT devices are usually extremely easy to use; nevertheless, people do need training, which also needs documenting, and this may be extremely difficult in environments with high numbers of potential users,

such as hospitals. Furthermore, the training must cover not only systematic routine operations and maintenance, but also pre-analytical factors, which can significantly affect sample quality and test results, and which are generally outside the knowledge of POCT users.

An additional and important challenge to effective use of POCT in many countries is inadequate regulation or legislation. Most developed countries do not currently regulate device selection, distribution, maintenance, user training, or results validation. Too often, laboratory professionals have no say in the use of POCT devices in their hospital, and may even be unaware of the number of such devices currently used by their colleagues. From my experience in the lab, there are many things that we can do to meet the challenges associated with advances in POCT technology and use. First, there is a clear need to proactively identify those specific clinical situations or healthcare issues that only POCT can improve. Selection of the most appropriate technology from the various options available is a crucial step in this process.

Additionally, not all devices marketed for a given application will have the same technical performance; we should not take their quality for granted. For example, Freckmann et al. report that a significant percentage of blood glucose

meter systems, even those carrying a CE label, do not meet the minimum accuracy requirements of the specific standard DIN EN ISO 15197 (1). Consequently, my feeling is that laboratories must be involved in the evaluation and selection of POCT devices that are to be used in local institutions.

Finally, we should be sensitive to the fact that some laboratory colleagues will see POCT as a threat to their jobs and to the status quo of their practice. Evidently, technological advances are enabling the development of new POCT devices and the decentralization of some tests from central laboratories to other settings. At the same time, other innovations are increasing test automation within central laboratories. This seemingly unstoppable process is characterized by small laboratories amalgamating with larger organizations, resulting in fewer overall laboratory jobs. Both of these trends, POCT and automation, will have a dramatic impact on our profession; under such circumstances, it is critical that we rise to the challenge and commit to leading the way in these changing times.

Reference

1. G Freckmann et al., “ISO 15197:2013 accuracy evaluation of two CE-marked systems for self-monitoring of blood glucose”, *J Diabetes Sci Technol*, 1, 6(5), 1060–75 (Sep 2012). PMID: 25901021.

Waging War on Pathogens

Molecular diagnostics are welcome reinforcements in the raging battle against institutional infection and antibiotic resistance



By Nancy Hanson, Full Professor,
Department of Medical Microbiology and
Director of the Center for Research in Anti-
Infectives and Biotechnology, Creighton
University School of Medicine, Omaha,
Nebraska, USA.

Very few procedures in modern medicine are possible without the administration of prophylactic antibiotics: chemotherapy, knee and hip replacements, heart surgery, and solid organ transplants all require treatment with one or more of these critically important drugs. However, the continuous rise in the frequency and range of antibiotic resistance is making the treatment and prevention of microbial infections challenging and in some cases impossible. The impact of antibiotic

resistant organisms on the treatment of patients is undeniable – the question is: how do we respond?

In my view, it is imperative that we broaden the techniques available to the clinical microbiology laboratory such that we can identify not only the infection-associated pathogens but also any resistance-associated molecular mechanisms they may possess. Taking a molecular approach can both improve the sensitivity and specificity of testing and decrease the time required to evaluate the pathogen and its mode of antibiotic resistance. Indeed, molecular testing has for many years been a mainstay for the identification of viral and bacterial infections, including methicillin-resistant *Staphylococcus aureus* (MRSA). These techniques are also relevant to today's major concerns – not least the detection of infections by those Gram-negative bacteria which are resistant to third generation cephalosporins and carbapenems by virtue of production of extended-spectrum β -lactamase (ESBLs) and carbapenemase enzymes. Given that β -lactams are the largest class of antibiotics prescribed today, the prospect of broad resistance to these drugs is singularly unwelcome. Accordingly, these bacteria have been recognized by the Centers for Disease Control in the USA and the World Health Organization as serious threats to human health. This threat is implicitly recognized in the recent approval by the US Food and Drug Administration (FDA) of two new antibiotics which work in combination as β -lactam/ β -lactamase inhibitors.

Molecular diagnostic approaches directed towards identifying the presence of ESBLs and carbapenemases have at least three benefits, as follows.

- Infection control – tests which identify the type(s) of β -lactamase producing organisms circulating in a particular hospital or community will assist the

development of infection control strategies.

- Antibiotic stewardship – tests which reveal the molecular basis of drug resistance, in addition to the drug susceptibility profile, will aid in selecting the best options for antibiotic therapy for a given infection.
- Monitoring resistance – tests which alert healthcare workers to the emergence of resistance mediated by a β -lactamase to a new or existing β -lactam drug will assist in developing a timely and appropriate response to such threats.

That said, not all of the >1,000 different β -lactamases demand routine application of a specific molecular diagnostic test. At present, the most clinically relevant β -lactamase genes are those that encode carbapenemases, ESBLs, and plasmid-encoded AmpC enzymes. Within this broad range of targets, a few are of particular importance. Thus, among the ESBLs, the CTX-M ESBLs – in particular, the CTX-M-15 and CTX-M-14 groups of enzymes – are the most prevalent group of ESBLs worldwide and also the most clinically significant. These are so commonly identified in isolates collected from patients in the community, mostly through urinary tract infections, that it is generally thought that no communities or hospitals are ESBL-free. Similarly, among the carbapenemase genes, we can focus on five families that are particularly important to target by molecular testing, namely the NDM, VIM, IMP, OXA-48, and KPC families. Last, but not least, are enzymes encoded by plasmid-located AmpC genes. Two of the six different families of plasmid-encoded AmpCs are particularly important: the CMY-2 and DHA families. CMY-2 enzymes are significant in that they are the most prevalent plasmid-encoded AmpCs in the world. DHA family members

“Adding molecular diagnostic tools into the workflow of the clinical laboratory will help to quickly identify any potential problems related to resistance, big or small.”

are also important since treatment with a third generation cephalosporin can result in the emergence of resistance to that treatment regimen. Since there are no recommendations by the Clinical Laboratory Standards Institute (CLSI) for identifying plasmid-encoded AmpC β -lactamases, I recommend a molecular test to identify the genes encoding these enzymes in species of *Enterobacteriaceae*.

It is critical that we identify the types of resistant organisms circulating in hospitals and communities and their respective mechanisms of resistance. The need for molecular diagnostics to detect and define the β -lactamases that underpin resistance is evident, but the most appropriate molecular tests will vary according to the specific needs of different hospitals and the different patient populations they serve. For all pathogens, however, molecular surveillance is a key component in combating resistance. Adding molecular diagnostic tools into the workflow of the clinical laboratory will help to quickly identify any potential problems related to resistance, big or small, and thus will allow the medical institution to quickly manage emerging issues and decrease the rate at which these types of organisms can spread in the community.



Animal Instincts

Meet the four-legged diagnosticians who are sniffing out cancer's secrets

By Michael Schubert

As far as working conditions go, Midas is probably one of the happiest laboratory employees there is. She works a five-day week, with no requirement to go in on weekends. She works for about 20 minutes at a time, with a maximum of an hour a day spent on the job. She's home by 3.30 pm every day. And, best of all, she gets praise, treats and rewards every time she does her job – namely, detecting cancer in urine samples. So how did Midas negotiate such a great deal for herself? It probably helps that Midas, a Hungarian wirehaired vizsla, is cute. It probably also helps that no laboratory instrument yet developed is capable of the sophisticated detection Midas is trained to do.

The idea that dogs can smell cancer has been around for quite a long time, although until recently, the claims were all unsubstantiated. Claire Guest, co-founder of the charity Medical Detection Dogs, shared an anecdote about a Dalmatian whose repeated licking alerted its owner to the presence of a mole on her calf that turned out to be malignant melanoma – a cancer that was unlikely to have been caught so soon by any other means. This was the story that caught Guest's attention and started her thinking about the potential implications. A psychologist working at Hearing Dogs for Deaf People, she was fascinated by canine behavior. Did cancer really have a smell? And if so, could dogs really detect – and possibly even diagnose – the disease?

Getting started

It was years later that Guest first heard John Church, the “maggot man,” interviewed on the radio. Church's claim to fame is the introduction of maggot treatment to the UK National Health Service, using medical maggots to remove infected or necrotic tissue from wounds. In his interview, though, he also mentioned that he'd heard a plethora of stories like the one Guest encountered, and that he was intrigued by the possibilities. Did anyone out there have the interest and expertise to train a “cancer dog,” he wondered? The answer was yes – and by the end of the day, Church and Guest were sitting together, making plans for the first-ever proof-of-principle study to see whether or not dogs could be trained to detect human cancers by odor.

But how could they begin training an ordinary dog to sniff out cancer? They needed a sample – something that would contain the odor of cancer, but not the cancer itself. After all, to a dog, a tissue sample is essentially a piece of meat. After weeks of deliberation, Guest and Church settled on testing urine samples from patients with bladder cancer; they knew that tumor cells are often shed into urine and can be detected via microscope, so they suspected it was possible that a volatile odor might also be present. The big question: could they teach a dog to detect it?

As it turned out, the six dogs they trained were able to



identify the urine of bladder cancer patients in 22 out of 54 cases – a mean success rate 14 percent higher than would have been expected by chance alone (1). One of the dogs was Guest’s own pet and cancer-sniffing pioneer, Tangle, who was able to detect cancer with 56 percent accuracy. In comparison with modern hospital tests, that success rate doesn’t necessarily inspire confidence – but the dogs had been exposed to only about 50 samples in total, half of which were positive. With a larger training set, the researchers were convinced the rate of detection would improve.

And they weren’t the only ones. Suddenly, attention was drawn to the fact that cancer apparently had unique volatiles that could be identified through odor. For Guest, it was the beginning of an era – one in which she envisioned that well-funded training programs would produce teams of biodetection dogs to save countless lives. Although in practice, engagement has been somewhat slower, significant steps have been made since that day: electronics scientists have begun to analyze urine components in search of detectable volatiles, there have been breakthroughs in “electronic nose” diagnostics, and researchers have continued to build a strong evidence base supporting

dogs’ noses as biosensors. Perhaps most significantly in Guest’s case, she and her first trustees set up Medical Detection Dogs and began a serious exploration of the possibilities of this off-the-wall, yet promising idea.

The nose knows

How good is a dog’s nose really? Humans have approximately five million sense receptors in their noses. Dogs, in comparison, have about 300 million – 60 times the sensitivity. “Some humans think they can smell a teaspoon of sugar in a cup of tea,” says Guest. “A dog with Midas’ nose would be able to smell a teaspoon of sugar in the equivalent of two Olympic swimming pools of tea.” And it’s not just the nose that does the job; dogs also have a vomeronasal organ, the organ of Jacobson, near the backs of their palates. When sniffing, they draw air into their throats so that it passes over this organ – which actually sends information to a different part of the brain. Sensory information from both the nose and the organ of Jacobson is consolidated in the brain before, ultimately, the dog makes a decision based on the scents it can detect.

Guest knew she needed to determine exactly how well the

dogs could smell – but the available literature varied widely. So rather than use “head space” (blowing air with volatiles into the dogs’ faces), she and her colleagues mimicked the technique they wanted to use by providing samples and asking the dogs to investigate them. “We wanted to find out how low the dogs could go,” she says, “and then I wanted to do some manipulation, by varying the temperature or how long the sample had been out, to see if I could improve their abilities.”

The dogs began by detecting amyl acetate diluted in mineral oil. The initial goal was to get them to “threshold,” the point at which they were experiencing a 50 percent success rate, before beginning manipulations – but the longer they worked, the better they got. No literature reflected that discovery, but experienced handlers had long suspected that, much like marathon training, the dogs needed to build up their abilities over time. Sure enough, the threshold point continued to decrease week after week, until after six months of training, the dogs were detecting at a threshold dilution of 1:5,000,000,000 – one part amyl acetate per half billion of mineral oil.

“Mass spectrometers are very good at finding specific things, but if you don’t know exactly what you’re looking for, it becomes much more complicated. Dogs are intuitive biosensors.”

But the real uniqueness of Medical Detection Dogs’ work came with their training system. Most systems are based on positive finds – dogs earn rewards for detecting something, but not if there’s nothing to detect. With cancer, though, the trainers didn’t know what the dogs were looking for, and they didn’t want to encourage a positive bias by rewarding the dogs only when they alerted. So they rewarded blank runs equally – resulting in a false positive rate of under 5 percent. That’s better than many medical tests, and at a fraction of the cost! At the moment, the charity is training a team of dogs to find prostate cancer; their accuracy using a 1 mL urine sample is over 90 percent, with a false positive rate below 5 percent. That means the biodetection dogs are actually performing better than prostate specific antigen (PSA) testing in the clinic (2)!

The demand for the dogs reflects their performance. “We’re already in a position where medics are asking us – can we send

“At one point, we had two very experienced dogs working on some samples that had come in from the hospital. The samples come with extensive patient notes, so we can see the Gleason scores, the PSA test results, and the clinician’s observations of the prostate.

We gave one sample to the dogs three times, and every time, they said no – there’s no cancer. But we knew the patient had a Gleason score of seven and the biopsy showed cancer. So we scratched our heads, tried again – and again, they all agreed no. Even after a third trial, the dogs just completely ignored the sample. So the clinicians went back to the notes – and they found that there had been a mistake and, by the time the urine sample had been taken, the patient’s prostate had been removed. There was definitely no prostate cancer in that sample! And then we said, my God, the dogs are good.”

“My dog, Daisy, was in a study screening prostate samples when she started to behave strangely around me. This lasted for a couple of weeks, and then one day I took all of the dogs for a walk. But Daisy kept jumping into my face and staring at me with these massive eyes. I kept pushing her off saying, ‘What’s the matter?’ And as she ran off I thought, ‘Oh she’s bumped into me; I can feel something,’” says Guest. “I kept an eye on it for a few days, and eventually it started to feel like there was a lump under there – so I went to my doctor and was immediately referred. At first, I was told that it was a cyst, but eventually I was diagnosed with breast cancer. I went through surgery and radiotherapy, but luckily didn’t need chemotherapy because my lymph nodes were clear. And I was told by my clinician, my oncologist and my surgeon that had my attention not been drawn to it, I wouldn’t have had a mammogram for a further five years and by the time I’d felt the tumor, it would have been very large and very advanced.

My story would have been completely different without Daisy. And it’s possible to apply this to people everywhere; every day of the week, we hear of people that have been diagnosed with or died of cancer. Early diagnosis saves suffering and lives, and that’s something our dogs can help give people.”



samples to your center?” says Guest. Medical Detection Dogs has a partnership with nearby Milton Keynes Hospital, which sends hundreds of samples to the charity for screening. Each sample is screened by a minimum of two dogs – usually three – to ensure accuracy and then the outcome is sent back to the hospital. Clinicians use those results in concert with symptoms, scans and PSA tests to decide whether or not to send patients for biopsies.

Bringing up biodetectors

What’s it like to train as a biodetection dog? “We have to teach the dogs when they’re young that this urine is basically a soup – it’s got volatiles, it’s got protein, it’s got everything – and the dog has to ignore about 98 percent of it completely.” Guest likens the process to training humans to pick up visual cues. “Imagine if I show you Monet paintings, and you get £100 for each Monet painting you spot with a small red flower in the left-hand corner. But I’m not going to tell you that; you’ve got to work out what it is in that painting that earns you the reward. Of course, the

more Monet paintings I show you, the better you get at it. To start with you’re just guessing!”

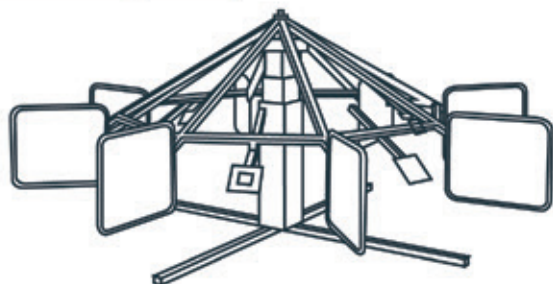
But what makes the dogs better than electronic noses or other detection devices? “A dog can recognize a ‘red flower’ even if it changes slightly – even if the wind is blowing it slightly to the left or there’s a yellow flower in front of it,” Guest explains. “The electronic machines aren’t quite so good at that. Mass spectrometers are very good at finding specific things, but if you don’t know exactly what you’re looking for, it becomes much more complicated. Dogs are intuitive biosensors; they can tell you that they see a red flower even though it may not look exactly like the red flowers they’ve seen before. And that’s what makes them particularly good at this work.”

Branching out

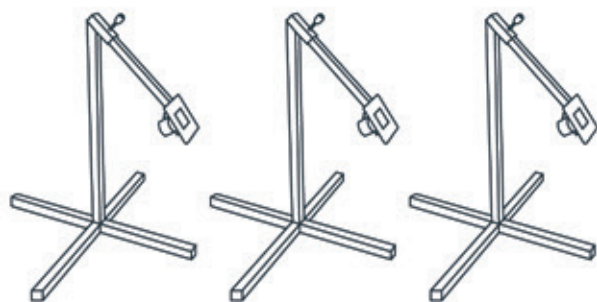
In 2011, the group published a second study (3). By that time, the highest-performing dogs showed sensitivities of over 70 percent, and the average dog could spot cancer in a sample two-

How does a dog screen patient samples for cancer?

The detection process



Eight samples are placed into the holder. The biodetection dog sniffs each one in sequence. If none contains the odor of cancer, the dog returns to the trainer – but if one does, then the dog will stop testing and alert to that sample. After alerting, the dog doesn't continue on to the rest of the samples; the holder is refilled and the dog starts again from the beginning.



These sample holders are often used for training or with less experienced dogs. A sample is placed into each holder and the biodetection dog sniffs each one in sequence. Just like with the multi-armed sample holder, the dog will ignore any samples that don't contain cancer, but stop and alert to any that do. After an alert, the sample holders are reloaded and the testing process restarted.

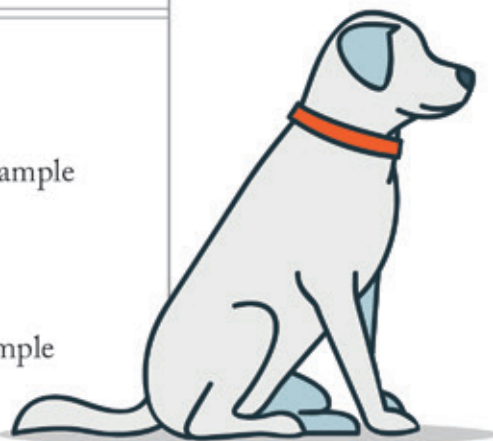
Dogs versus machines

Dog's nose

Speed	Two seconds per sample
Sensitivity	1:500,000,000
Working hours	Up to 1 per day

Electronic nose

Speed	10 minutes per sample
Sensitivity	1:50,000,000
Working hours	Unlimited



How does a dog alert?

Each animal is different. Some sit; others stand and stare; still others gesture or point with their feet. Handlers get to know each dog and recognize their different alert signals.



How long does a sample last?

About half an hour at room temperature or a little cooler. After that time, the odor of cancer dissipates and the sample is no longer usable.

How long does it take to fully train a dog?

About six months. Each trainer works with four or five dogs at a time for a maximum of one hour a day.



What if a sample isn't a straightforward yes-or-no?

Medical Detection Dogs and the Open University are collaborating on a new device that includes a pressure plate, a light beam, and specialized software that allows the dogs to provide not just binary, but quantitative information.

“Just before they went to double-blind testing for our first study, the hospital urologist came to see the dogs working. All of the samples were new ones that the dogs had never seen, and one dog – Tangle – kept on indicating a particular sample. I couldn’t train him not to respond to it. I tried everything – giving him massive rewards, putting a positive cancer sample next to it to get him to pass over the normal one and indicate the cancer; he just kept going to this sample.

Eventually, he started to get quite fed up about it. The consultant was ready to move to double-blind, but we had a major problem with our best dog.

So we took the sample out and showed it to the clinician, and he pointed out that the response was the same as the one for bladder cancer. I said, ‘I know, but you’ve told me this man’s bladder is completely clear.’ He went back, looked at the notes, realized that the patient hadn’t had an ultrasound, and found that he had cancer of the kidney. About three weeks later, we got a letter from him saying, ‘Thank you, dear Tangle; you saved my life.’ His cancer was in the epithelial lining of the kidney, so it was part of the same system as the bladder. To Tangle, without any training, it was exactly the same,” explains Guest.

thirds of the time. What’s more, the results weren’t specific to a type of cancer; dogs trained on bladder cancer samples and then presented with prostate cancer reacted in very similar ways. “When they went up to the prostate cancer samples, they went, ‘Oh, yes, I think that’s it, but not quite.’ You have to reward them a couple of times before they’re certain. So I think there’s a big part of the odor that’s the same, but not all of it.” At the moment, Medical Detection Dogs has three separate sets of biodetection animals in training for bladder, prostate and kidney cancers – so that if a dog alerts, consultants will know to look for that dog’s particular specialty.

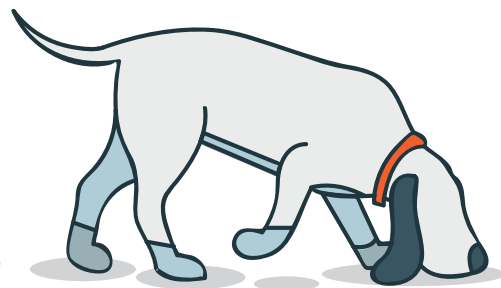
As engagement increases, Medical Detection Dogs is expanding the animals’ repertoire even further. Their sights are now set on breast, ovarian, colorectal and even lung cancer. The latter two are particularly exciting. Lung cancer, responsible for almost one in five cancer deaths, is the most common cause of cancer death worldwide (4). Over three-quarters of lung cancers are diagnosed at an advanced stage (5), and the charity is optimistic that, with a new breath test in development, the dogs will be able to improve

early diagnosis of lung cancer in the same way that they’ve shown an ability to detect early bladder and prostate cancers. Colorectal cancer detection is equally useful – the disease is the second-most common cause of cancer death in Europe and the fourth-most common worldwide (6), and over half of patients are diagnosed at an advanced stage (7). “I asked the colorectal surgeon, ‘Do we really need better testing for this cancer? You have stool screening – don’t they know if you’ve got blood in your stool?’” says Guest. “He said that most people die of colorectal cancer because they won’t do the stool sample. There’s much higher resistance to doing a stool sample than a urine sample. And people are dying because of it. So if we could detect colorectal cancer in a urine sample, a lot more people would be screened.”

Biodetection dogs clearly have potential. Although there’s always a need for more funding, more studies and more evidence, these hardworking animals have already carved out a niche in the world of early cancer diagnosis – and the results keep on coming. As Medical Detection Dogs expands its remit to include more diseases both within and outside the cancer sphere, it looks like the dogs are here to stay. Could pathologists one day share their laboratory space with furry, four-legged disease detectors? It seems unlikely, but you never know...

References

1. *CIM Willis et al., “Olfactory detection of human bladder cancer by dogs: proof of principle study”, BMJ, 329, 712 (2004). PMID: 15388612.*
2. *MJ Barry, “Clinical practice. Prostate-specific-antigen testing for early diagnosis of prostate cancer”, N Engl J Med, 344, 1373–1377 (2001). PMID: 11333995.*
3. *CIM Willis et al., “Volatile organic compounds as biomarkers of bladder cancer: Sensitivity and specificity using trained sniffer dogs”, Cancer Biomark, 8, 145–153 (2010–2011). PMID: 22012770.*
4. *Cancer Research UK, “Lung cancer mortality in Europe and worldwide”, (2014). Available at: <http://bit.ly/1qdLRjv>. Accessed May 11, 2016.*
5. *Cancer Research UK, “Lung cancer incidence by stage at diagnosis”, (2015). Available at: <http://bit.ly/1WpalEp>. Accessed May 11, 2016.*
6. *Cancer Research UK, “Bowel cancer mortality in Europe and worldwide”, (2014). Available at: <http://bit.ly/1ZFdIVU>. Accessed May 11, 2016.*
7. *Cancer Research UK, “Bowel cancer incidence by stage at diagnosis”, (2015). Available at: <http://bit.ly/1JZSvQN>. Accessed May 11, 2016.*





AN INTERVIEW WITH CLAIRE GUEST

What role do volatiles play in disease diagnosis now?

Interest in volatile detection in cancer has grown hugely since our 2004 paper (1). It's vital to develop a good evidence base, but that requires clinical trials. It's a chicken-and-egg problem, because you have to start small and do a proof-of-principle study. Then you can do a larger trial with more patients, and if that's successful, you can do multicenter studies to examine things like sensitivity and specificity. The challenge for us has been moving from the small proof-of-concept studies – which have had a lot of interest, but also invite skepticism because of the small sample size – to trials with a much larger sample size that yield a stronger evidence base. That's what Medical Detection Dogs has spent the last decade doing, so we were pleased recently to get ethical approval for a breast cancer study, and for a three-year study on prostate, kidney and bladder cancer.

That one is a very significant study, because we're not only looking at the dogs' sensitivities and specificities over much larger sample sizes, but also observing them longitudinally. Patients in active surveillance come back every six months for check-ups, and every time a clinician sees the patient, a dog examines a sample. So we'll be monitoring the progress of the dogs and the patients together, and we'll be able to see whether the dogs can indicate the presence of cancer earlier than standard methods. That's important because prostate biopsy currently has such a high false-negative rate; we need a more reliable test.

I think the detection of human disease by volatiles has largely been forgotten, although it was something that people talked about historically. In other parts of the world where testing isn't as advanced as ours, people still talk about it. We're not saying that our dogs are the ultimate answer for every disease and condition – but we are saying that there's a huge area of diagnostics that is being overlooked, and our dogs are leading the way.

How might volatiles affect the way a diagnosis is achieved?

For every disease where early diagnosis is a struggle, detection through associated volatiles could become part of the diagnostic process. It may be for some conditions, that associated volatile pattern may be relatively straightforward to uncover once you know it's there. For instance, the fact that a dog might detect a particular early-stage disease with 95 percent reliability on a skin pad tells scientists that the volatile pattern is easy to find, occurs before symptoms are seen, and can perhaps be detected

with an electronic nose. Ultimately, that knowledge translates into a new diagnostic instrument for the disease.

In other conditions, the pattern might be much more difficult to detect. It might take a decade or two for a machine to do it reliably – and in the meantime, dogs could continue to screen those samples for physicians to use as part of the diagnostic picture. At this point, we're not talking about screening populations; we're testing symptomatic individuals, so it's a small group of patients rather than thousands of samples a day. Later on, there's a possibility that we could expand to screening high-risk groups (for instance, people who are predisposed to lung cancer and who are coughing), but there simply aren't enough trained dogs to go out and screen healthy populations right now.

Why wouldn't everyone want a canine colleague in the lab?

That's a very good question. Some healthy skepticism at the beginning of our investigation was appropriate. But I think that, if we continue to produce this strong evidence base, then we have to ask: If this work is saving lives and helping patients avoid the painful and invasive treatments that come with late-stage disease, why wouldn't you want to work with biodetection dogs?

The way we envisage it – and we have plans to develop this in the future if our next three years are successful – is that we'd have a hospital that included a patient-free area where a handler and a dog would come in on a daily basis and screen samples. That way, we could also use dogs to spot diseases where speed matters – for example, highly infectious conditions where you don't want the patient to leave without a diagnosis in case they spread the disease. That's been very successful with African pouched rats in Tanzania. The rats test sample for tuberculosis, and they spot patients that would otherwise rejoin the population and pass on the infection. We think it's possible that similar programs could be instituted for sexually transmitted diseases and other infections.

But in order to do any of this work, it has to be funded – and medical economics can be quite complicated. Recently, numbers have begun to emerge showing that early cancer diagnosis saves money because of the high cost of therapy for advanced disease. And I think that's the important point. Reliable early diagnosis allows us to treat diseases before they become hugely complicated and expensive. We could improve current diagnostic methods, too; screening tests and biopsies cost clinicians and patients time and money – and if the tests have a high false-positive rate, it's not time and money that's being used wisely. I think that's where our dogs come in... to help identify the right patients at the right time.

the Pathologist

Sign up
now for
FREE

Website

The website acts as a hub for all content and our community – join the discussion by leaving comments.

Print

The printed version is free of charge in both Europe and the USA – subscribe to guarantee your copy.

App

The iPad edition offers an engaging multimedia experience – download it for free from the App Store.



Digital Magazine

The PDF version replicates the print issue – share the latest issue easily with colleagues.

Optimization for Mobile

Content is optimized for handheld devices – access content anywhere, any time.

Social Media

Our social media channels allow quick and easy dialog – engage with us!

To subscribe go to www.thepathologist.com/subscribe

Or email tracey.nicholls@texerepublishing.com



In Practice

*Technologies and techniques
Quality and compliance
Workflow*

30–33

People versus Machines?

Microbiology laboratories can stay open longer and increase efficiency thanks to automation – but not without the contributions of highly trained staff.

34–36

Disrupting Cytogenetics in the Clinic
Mate pair sequencing, when paired with appropriate protocols and algorithms, offers a cheaper and less data-intensive alternative to whole-genome sequencing.

People versus Machines?

Intelligent incorporation of automated technologies into pathology laboratories will improve service provision by complementing – not replacing – a highly skilled workforce

By Simon Rattenbury

For years, microbiology laboratories have performed many of the same tasks – microbial detection, identification, resistance testing, and a host of similar applications involving test tubes, flasks, and above all, time. Microbiology is a resource-intensive discipline, which can cause problems when labs simply don't have the necessary equipment, staff or skills. One solution that improves year on year is laboratory automation – letting machines assist with, or even independently handle, some of the tasks that are tricky or take additional time to do by hand. But how can pathology labs access this kind of technology?

At a Glance

- Although it has been used for some time in other disciplines, automation is a recent entry into the race for better microbiology
- Not only can it take the pressure off strained staff, but it can also provide certain answers that are faster or more accurate than the manual equivalents
- It can't be used in isolation, though; human beings must review and assess the results of automated laboratory tests
- Only with thoughtful application of automation, continuous support by industry, and appropriate investment in the human workforce can microbiology services improve

How can they make best use of it? And how can they ensure a balance between automated contributions and the “human factor?”

As a healthcare scientist, I have four decades of experience in both the public and independent sectors. For the last 18 years, I've been head of the microbiology service at the Royal Free Hospital (RFH) in London – a responsibility that includes the high-level isolation unit pathology laboratory in which we process samples from patients with viral hemorrhagic fever. I've also held a variety of other pathology-related roles, from laboratory information management system (LIMS) implementation to project management. Recently, pathology at the RFH has entered into a joint venture in which I am the scientific lead: we've merged microbiology services for two separate London National Health Service (NHS) Trusts in concert with an independent provider. The result – Health Services Laboratories – is our way of concentrating expert services on one site, with the goal of improving patient services and more efficiently using NHS resources.

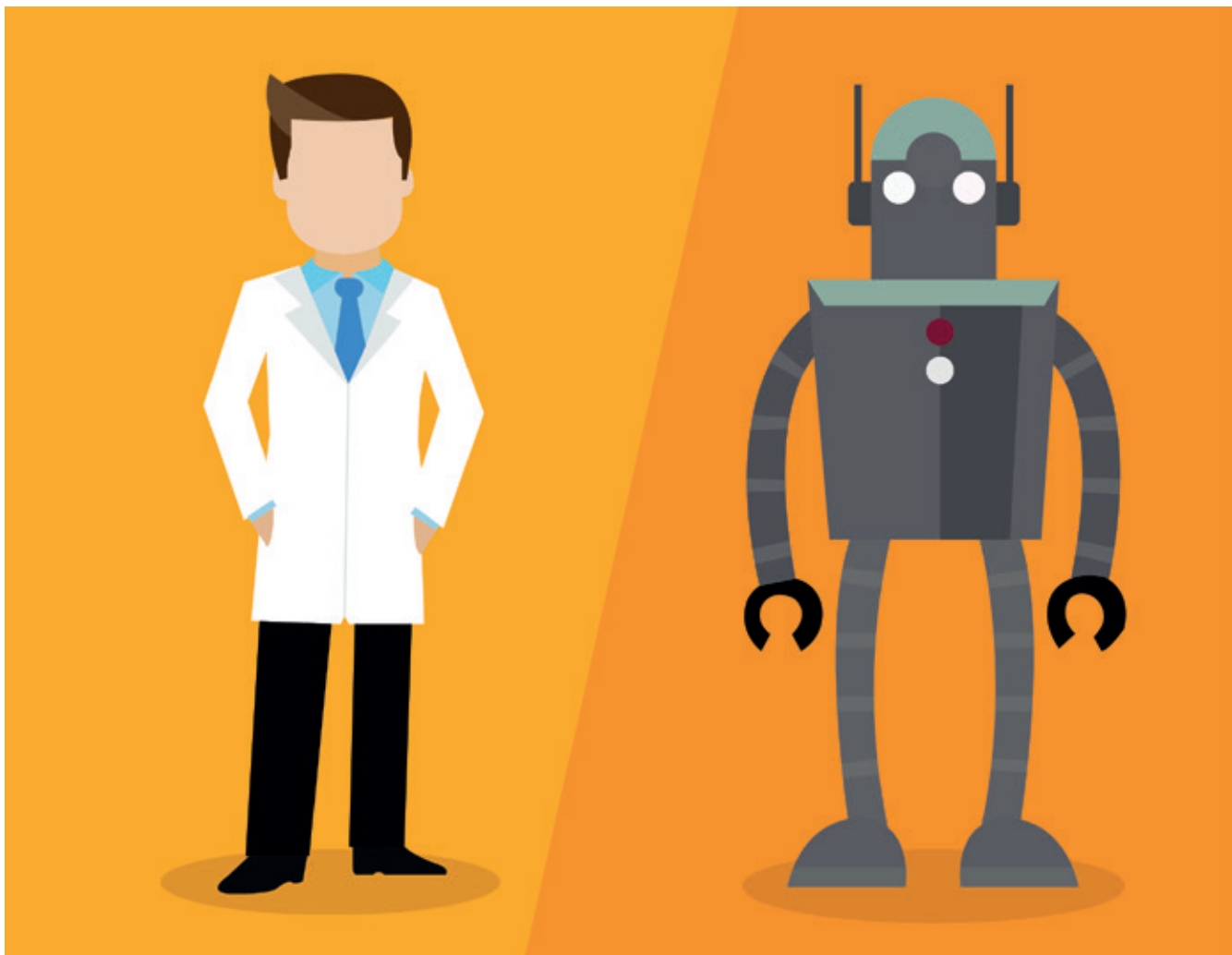
Thanks to the service reconfiguration, we now have a lot of intriguing new opportunities. My colleagues and I have been working together to develop a clinically-led model for the future of infection services within Health Services Laboratories. Our laboratories are new and state-of-the-art, providing us with an environment in which best practice can be achieved. But most of all, we're excited about the future development of molecular and automated sciences – advancements that will let us focus our attentions on the areas of greatest need (for instance, antibiotic resistance or syndromic medicine and surveillance) to improve outcomes for our patients.

Historical obstacles

Until recently, microbiology hasn't had

the benefit of significant automation like the kind we've seen in clinical biochemistry or hematology. Instead, it has relied on well-educated, highly trained staff to first process patient samples and then read hundreds of cultures and antibiotic sensitivity plates! Traditional microbiology testing generally includes microscopy, antigen detection, serology and culture – and, where possible, we've tried to introduce automation with the use of multipoint inoculators, microplate readers, and antibiotic disc readers. In the case of bacterial and fungal infections, clinical specimens are inoculated onto a range of selective and differential media, then incubated at various temperatures and atmospheres. In most cases, biochemical testing is then performed to identify species, and plating or broth dilution is used to determine antimicrobial susceptibilities. The only rapid, reliable method available is Gram staining – which, even now, results in a 17 percent decrease in mortality when the results are reported in under an hour (1).

Unfortunately, not all of these tasks have historically lent themselves to automation. As a result, staffing is a major and costly issue within pathology, because we need to ensure that sufficient levels of qualified and support staff are available around the clock for timely patient care. In certain areas of the United Kingdom – like London, where I work – that can be a challenge, because it's difficult to employ large numbers of staff due to the high cost of living; in other areas, there might be different problems, such as few trained staff available or a lack of resources to out-compete larger laboratories. In theory, staff numbers can be reduced if we increase our use of automated platforms, but here, too, there's a caveat; automation is only as good as the quality of staff operating the service, as it's their job to ensure that the systems are verified,



validated, quality-controlled, and that the results make sense in clinical context.

Traditional microbiology cultures were thought to represent a “gold standard” of diagnosis, but with today’s technological advancement, that’s no longer really the case. In fact, their sensitivity and specificity are poor for many reasons (the distribution of organisms in patient samples, poor sampling and transport techniques that could kill microorganisms, misidentification, and even organisms like *Chlamydia* species that can’t be cultured in a routine setting) when

compared to modern nucleic acid amplification techniques. Phenotypic testing presents a challenge in terms of identification to species level, whereas 16S rRNA gene sequence comparisons allow universal phylogenetic tree comparisons. Even antimicrobial sensitivity testing results can vary based on the media used, atmospheric conditions, and the person reading the end result.

One significant drawback trumps all of the others, though: the time to result, or turnaround time. In some circumstances, traditional testing may

take days or even weeks to return results – and in the case of organisms like mycobacteria or fungi, a delayed or incorrect diagnosis can result in inappropriate antimicrobial prescription and adverse effects for the patient, which could be life-threatening! In severe disease, even an hour’s delay in treatment may significantly affect the outcome. We need testing methods that are not only accurate, but fast – because ensuring that patients receive the right drug at the right time is good both for individual outcomes and for antimicrobial stewardship.

Tackling preconceptions

It seems clear that the benefits of automation are in demand, which explains why, since the mid-2000s, companies have worked hard to introduce automation packages to suit most diagnostic environments – sample spreaders, antibiotic sensitivity and agar plate readers, and an ever-increasing array of nucleic acid amplification analyzers and sequencers.

“Even more important is avoiding the “black box” mentality – the belief that a department can use automation to reduce skilled personnel numbers or replace them with staff who have only basic scientific training. This is fraught with ignorance.”

Automation doesn't mean just pushing thousands of agar plates around a system that incubates them and takes images. It's much more than that – a combination of methods and science. So how can it help overcome the issues with traditional

testing? The answer to that question depends on the type of automation, what's incorporated into the system, and to what degree the methods are applied directly to patient samples. Some well-known applications for detecting pathogens directly from the patient use simple lateral flow devices or high-throughput molecular systems to detect chlamydia, gonorrhoea, methicillin-resistant *Staphylococcus aureus*, fecal pathogens, or dermatophytes from patients' nails. All of those tests yield results within a few hours and can be automated for even faster readouts. But that's not the only area in which automation can help. To offer just one more example, it undoubtedly improves the diagnosis of sepsis. The use of blood culture monitoring systems in combination with MALDI-TOF mass spectrometry, rapid fluorescence in situ hybridization, and automated minimum inhibitory concentration (MIC) sensitivity testing, enables a meaningful result in eight to 12 hours (compared with the 48 to 72 hours of conventional testing). The outcome? Rapid treatment and reduced morbidity and mortality.

Central to automation is information technology (IT). From hospital order communications to LIMS, it's the key element that bonds the individual components of automation together. In concert with expert rules, IT can help users enhance data flow – and, in some circumstances, even complete tasks with no intervention from medical staff. For instance, computers can apply expert rules so that isolates from specific, fully sensitive sample types can be authorized directly, while isolates that fail the expert rule are directed to the medical team for further investigation. With the advent of the smartphone, patient data can be sent directly to the clinician via the LIMS, saving time and money in antibiotic stewardship. But human beings aren't the only things for which IT reduces the need; another benefit is that it's usually a paper-light, or even paper-free, system.

In our microbiology service, we currently use a variety of automation options. We perform automated blood cultures, antibiotic sensitivity testing, microbial identification using MALDI-TOF, molecular pathogen detection (for MRSA, CRE, *Clostridium difficile*, chlamydia, gonorrhoea, tuberculosis, other mycobacteria, enteric pathogens, and dermatophytes) and 16s 18siITC sequencing. Wherever possible, all systems are interfaced using either our own LIMS or the integration engine associated with the machines. At the moment, we're anticipating an upcoming move to a new, state-of-the-art center for infection, at which point we will be stepping up our use of automation, making strategic use of a KIESTRA lab automation system (BD) to deal with a complex workload from a range of clients.

Automating appropriately

Despite automation's many advantages, potential users should remain mindful that all that glitters is not necessarily gold. It's important to carefully consider the patient population and the microbial epidemiology when bringing the component parts together, ensuring that each test's positive and negative predictive values meet your laboratory's needs. Even more important is avoiding the “black box” mentality – the belief that a department can use automation to reduce skilled personnel numbers or replace them with staff who have only basic scientific training. This is fraught with ignorance – after all, just because the system produces a result doesn't mean that the result is correct. For instance, if we examine nucleic acid amplification testing and threshold cycle (Ct) values, where this is the intersection between an amplification curve and a threshold line, we get a measure of the concentration of target in the polymerase chain reaction. But many factors besides that concentration impact the absolute Ct value. As human beings, we can untangle

those complicating factors to understand the test output. But in automated testing, does the software interpret the Ct values correctly? We need a skilled laboratory professional to verify that – because if it doesn't, and there's no operator looking at the curves, then as with any automated tool, we may end up with a result that is meaningless, or worse, incorrect in a way that places a patient at risk.

Automation isn't one-size-fits-all, either. The size and scope of the laboratory can influence the type of system that can be used. The footprint for high-throughput analysis, for instance, is not small, especially in circumstances where more than one analyzer is needed. Does your laboratory's infrastructure have room to accommodate all the necessary workflows of best practice for molecular diagnostics while minimizing environmental contamination? Not every service can make the jump to automation in the same way, or with the same tools.

Investing in people

It seems counterintuitive to say that people are the most important aspect of automation, but it's also true. The key to an efficient laboratory is getting the skill mix right. That means not just using staff at a basic grade, but investing in the workforce. Hiring the right people is the obvious first step, but what's next? Providing continuing professional development keeps healthcare scientists up to date so that they can develop and troubleshoot equipment and assays. Employees with growth opportunities stay motivated, and motivated staff take less time off and make fewer errors. In an ideal situation, an automated laboratory should be well-staffed, run 24/7, accommodate the vagaries of molecular workflow, and incorporate automated microbial culture so that samples flow through the system without the need to batch additional tests like MALDI-TOF or antimicrobial resistance detection. But a system like this comes

at a price, and with the NHS and so many other healthcare systems financially stretched, that's more of an obstacle than ever. How can we overcome it? Healthcare scientists and clinicians must work together to demonstrate that automation offers better patient outcomes, more efficient bed management, fewer nosocomial infections, and a reduction in the use of expensive antimicrobials.

Importance of industry

People are the main drivers of the future of automation, but others – industry, innovation, and point-of-care testing opportunities – also play a role. MALDI-TOF mass spectrometry is a great example of the way industry plays into advancing automation. The technique has revolutionized microbial identification by increasing speed and accuracy, forcing manufacturers of phenotypic methods to rethink their approaches to keep up. Convenience is also important; inevitably, patients and clinicians require diagnostics as close to the patient as possible, so the use of smart devices and the ability to monitor patient parameters in real-time are major incentives for development. My hope in that regard is that we'll soon improve our ability to distinguish colonization from true infection. At present, we culture sputum for so-called pathogens – but we know that sputum is a poor sample to use for diagnosis, because it's possible that the organism we detect is only colonizing the respiratory tract. I think the solution to this problem, and to many of automation's weaknesses, lies in industry. Diagnostic companies should be developing automated systems that can detect pathogens with resistance profiles directly from patient samples by applying a combination of molecular detection and mass spectrometric quantification in real-time. In fact, I suspect that this may well be the future of the diagnostic microbiology laboratory!

As our financial and technological situation changes, the landscape of pathology is changing along with it. Wherever possible, it's important for local departments to work together, sharing equipment and resources so that we can all move toward fully staffed, 24/7, best-practice service together. Automation is an increasingly significant part of that. In my opinion, it has already improved patient outcomes, at both relatively simple levels of automation like using software to reduce transcription errors, and more complex aspects like using mass spectrometry and NAAT to reduce the time needed to detect a pathogen from days to hours. But the biggest benefits come from applying science wisely. There's no point investing in automation if the science isn't focused on the most vulnerable, immunocompromised patients. Why? That's the population that generally has the worst outcomes and costs service providers the most money through prolonged stays and expensive treatments.

With the emergence of totally treatment-resistant bacteria, and the rapid evolution of new diseases and new drugs, it's vital that we use the best science available to us to reduce the spread of these organisms. With services thoughtfully designed to incorporate the careful use of automation, I think we'll find ourselves much closer to the solutions we seek.

Simon Rattenbury is Head of Laboratory Service in HSL Microbiology at The Royal Free London NHS Foundation Trust and scientific lead London for Health Services Laboratories, UK.

Reference

1. KA Bauer et al, "An antimicrobial stewardship program's impact with rapid polymerase chain reaction methicillin-resistant *Staphylococcus aureus*/S. aureus blood culture test in patients with S. aureus bacteremia", *Clin Infect Dis*, 51, 1074–1080 (2010). PMID: 20879856.

Disrupting Cytogenetics in the Clinic

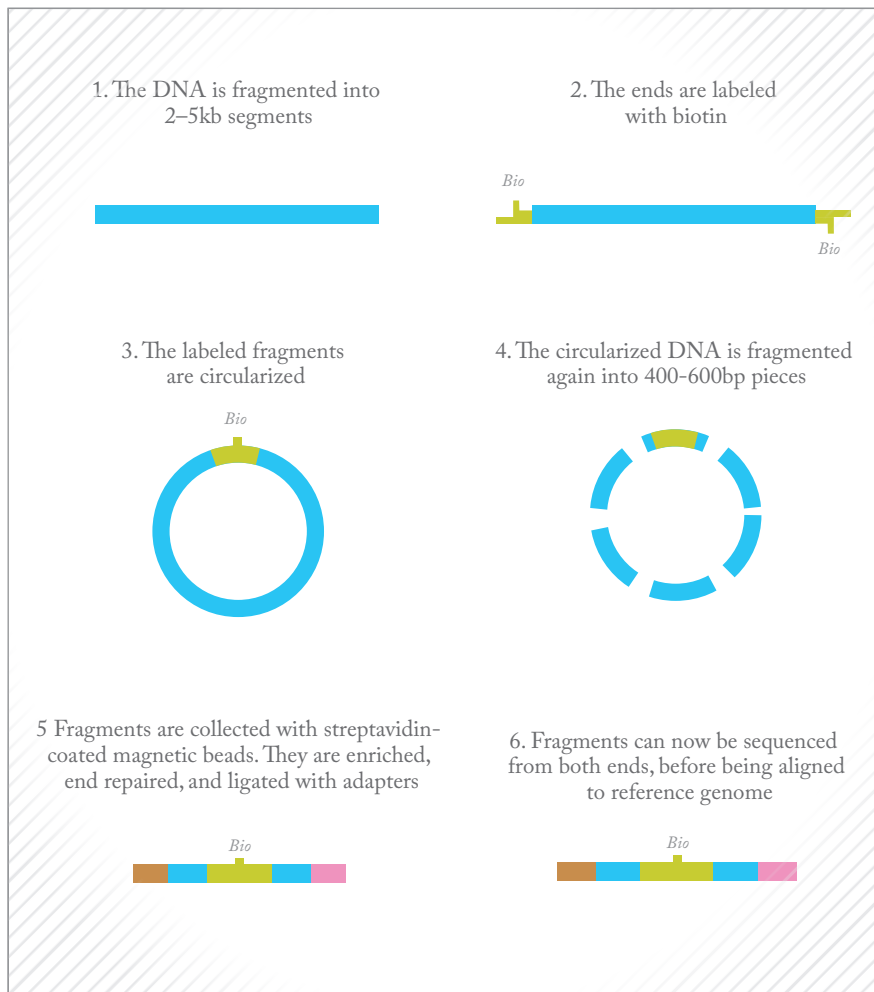
To select the right treatment for each cancer patient, we need fast, accurate and cost effective ways to characterize tumors. Now, with newly developed algorithms and protocols, mate pair sequencing could well be the tool we've been seeking

By George Vasmatazis

The entry of next-generation sequencing (NGS) into clinical practice has been disruptive. We can now analyze many more samples, with much less money, and in more depth than ever before, allowing us to comprehensively interrogate the entire genome of cancer cells. In the past, we could only assess one known gene at a time, but now NGS allows us to look at the entire genome in a single assay – completely changing how we do translational research and how we

At a Glance

- Next generation sequencing techniques have revolutionized the way we assess many disorders in the clinic
- However, we still need a sequencing solution that is manageable in terms of both cost and information volume
- We have developed new algorithms and protocols for mate pair sequencing – which, at under US\$1,000 per genome, may be the solution we need
- The next challenge is to find ways of displaying the resulting data so that the test's end users – clinicians, researchers and pathologists – can access it quickly and efficiently



Mate pair library preparation.

will do clinical genomic testing.

Traditionally, we have taken a bottom-up approach in biomarker discovery. Basic scientists look at an interesting pathway in the cell that may be associated with tumor behavior. They find a limited number of genes or proteins related to that pathway, and study them to find out how they work, and whether they might have potential as biomarkers. Along with John Cheville, I direct the Biomarker Discovery Program at the Mayo Clinic's Center for Individualized Medicine. Here, rather than starting from a gene or protein of interest, we start with a practical clinical question that fills a

physician and patient need, and aim to identify a biomarker and develop a test to answer that clinical need. We refer to this process as product-driven biomarker discovery. Economically, we believe this makes a lot of sense – the clinical need dictates the experimental design, validation and assay development, rather than a more random approach.

The search is on

At the DNA level, cancer acquires mutations and small indels (insertions or deletions of bases) or large chromosomal rearrangements. A lot of investment has gone into investigating point mutations

but, around seven years ago, I decided to turn our attention towards large chromosomal alterations, which occur in many cancers and are important in determining cancer behavior and response to treatment. But how can you best identify those alterations? One approach is whole-genome analysis, but at the moment this would cost more than \$10,000 per patient. And before you can find any commonalities among patients, you have to do hundreds of samples, so that whole-genome analysis quickly becomes too expensive. It also generates a huge quantity of information that we don't always know how to handle, and produces a lot of "noise". And as the data grow and grow, we need faster and better algorithms, which can be a challenge to create.

To solve this problem, we developed a new strategy based on Illumina's mate pair sequencing. Mate pair sequencing allows us to look at the whole genome of tumor cells for rearrangements, deletions, amplifications, and gains – all for less than \$1,000 per sample. Our protocols and algorithms make the technique viable for routine clinical use. Typically, if you contaminate a tumor sample with too many normal cells, you lose the signal from the cancer cells. So we have developed protocols for laser-capture microdissection to obtain a very pure population of cells. In addition, we developed bioinformatics algorithms we call BIMA and SVA tools that filter out the "noise" and significantly reduce false-positive results. In brief, BIMA handles sequencing artifacts inherent in mate pair library preparation, including biotin junction reads, paired-end read contamination, chimeras, and so on. With these new protocols and algorithms, mate pair sequencing is being implemented in our clinical laboratories, where we believe it will replace 95 percent of conventional cytogenetic testing. For instance,

the complex multiple tests applied to bone marrow samples in patients with leukemia can now be done with a single mate pair sequencing assay – faster, cheaper and comprehensive (1,2).

“They have to look at a totally different kind of dataset to understand what’s going on with the patient. That’s certainly a challenge, but if anybody can do it, it’s pathologists. No other medical experts know as much about what happens in a cell.”

The tradeoff for the broad coverage and low cost of mate pair sequencing is that it has limited sensitivity, so there will still be a role for more sensitive assays such as fluorescence in situ hybridization (FISH). It's a little like Google Earth – mate pair sequencing allows you a bird's eye view of the entire genome but if you want to see all the details you have to focus and zoom. We envision that mate pair sequencing will be the "go to" test, with karyotyping, FISH, and arrays being used as needed for confirmation or follow-up.

Sequence, and ye shall find

The first clinical oncology application is likely to be in hematological cancers, in which rearrangements and gene fusions are already being used as biomarkers to aid diagnosis and direct treatment. In this case, mate pair sequencing can simply be incorporated into existing protocols to provide a more cost-effective means of detection.

More challenging, but very exciting, is our ongoing work on solid tumors. There are several important clinical questions that we believe mate pair sequencing can help resolve. For example, the majority of men will develop some form of prostate cancer as they age. In most cases the disease will be slow growing and require no treatment, but some will be much more dangerous, fast-growing tumors, with a risk of metastasis. Currently, it is difficult to tell the two types apart, leading to unnecessary treatment for many men. We are using mate pair sequencing to search for genomic markers that can separate clinically insignificant prostate cancer from more aggressive prostate cancer that requires treatment (3,4).

Lung cancer is another area of interest for us (5). Some patients present with more than one tumor in their lungs; it is important for physicians to know whether this is the result of one tumor that has metastasized or two separate primary tumors. Gene rearrangements are common in lung cancer (for example, the fusion of *EML4* and *ALK* to form the *EML4-ALK* oncogene) and tumors of common origin will have identical rearrangements and breakpoints. Using mate pair sequencing, we are able to detect similarities and differences in the rearrangements of the two tumors, and determine if they are related. These results will determine if the patient is a candidate for curative surgery for two independent primary tumors or should receive chemotherapy for metastatic lung cancer.



Making it easy

What will that look like for cytopathologists in the lab? I don't think morphological pathology is ever going to go away – we need people who know which cells to interrogate – but it's clear that we also need molecular information. I anticipate that morphologically trained pathologists will have to learn molecular pathology as well. Most of them are already familiar with some of the techniques, but the new way of doing things is on a completely different scale. Molecular pathologists usually work with one or two proteins, maybe a panel at most. Now we're talking about putting a whole genome into their computers at once. They have to look at a totally different kind of dataset to understand what's going on with the patient. That's certainly a challenge, but if anybody can do it, it's pathologists. No other medical experts know as much about what happens inside a cell.

The people who will be using these tests – clinicians, researchers and pathologists – understand the disease and its genetics very well. What they don't necessarily

understand is data. And the data that results from mate pair sequencing is completely different to the FISH panels or chromosomal bands that they are used to. A bigger challenge for users is that the technologies they used in the past gave them access to a relatively small amount of information. Today, whole-genome technologies, such as mate pair sequencing, are likely to result in information overload. And it's not just too much information, it's too much important information. The next big challenge for us is to create visualization techniques to transform data into something that can easily be understood by clinicians and patients.

Genomic research programs are expensive enterprises, so we need a return on investment if we are to continue our work. But, for me, financial reward has never been a motivation – our primary goal is to improve patient care. And it's clear that genomics – using the right tools – will play an important part in achieving that. It is a very satisfying feeling to know that we have helped

drive mate pair sequencing into the clinic to improve patient care.

George Vasmatazis is co-director of the Biomarker Discovery Program within the Center for Individualized Medicine at the Mayo Clinic, Rochester, USA.

References

1. G Vasmatazis et al., "Genome-wide analysis reveals recurrent structural abnormalities of TP63 and other p53-related genes in peripheral T-cell lymphomas", *Blood* 120, 2280–2289 (2012).
2. AL Feldman et al., "Discovery of recurrent t(6;7)(p25.3;q32.3) translocations in ALK-negative anaplastic large cell lymphomas by massively parallel genomic sequencing", *Blood* 117, 915–919 (2011).
3. SJ Murphy et al., "Mate pair sequencing of whole-genome-amplified DNA following laser capture microdissection of prostate cancer", *DNA Research* 19, 395–406 (2012).
4. IV Kovtun et al., "Lineage relationship of Gleason patterns in Gleason score 7 prostate cancer", *Cancer Res* 73, 3275–3284 (2013).
5. SJ Murphy, "Identification of independent primary tumors and intrapulmonary metastases using DNA rearrangements in non-small-cell lung cancer", *J Clin Oncol* 32, 4050–4058 (2014).



NextGen

*Research advances
New technologies
Future practice*



38-41

Pathogens Unveiled

Every physician knows viral infections don't need antibiotics – but it can be hard to tell which pathogen is causing a disease. Could specific “gene signatures” be the answer?

42-43

Motion Pictures

Current lung tumor imaging methods allow us to capture either the detail or the movement of the tumor – but when both are needed, MRI+CT fusion may help.

Pathogens Unveiled

Researchers are on a mission to develop rapid point-of-care tests to establish infection cause and drive down unnecessary antibiotic use

By Ephraim Tsalik

When patients come in with respiratory complaints (like coughs, sore throats, or runny noses), physicians go through a typical diagnostic process to figure out the source of the problem. Most of the time, symptoms like these are due to infections – but that’s not always the case. For example, allergies can present identically to a viral infection. Congestive heart failure can sometimes be hard to distinguish from pneumonia. Other inflammatory conditions can also adopt a very similar appearance to infection. So even the most basic

At a Glance

- *Patients with respiratory infections are often prescribed antibiotics, even when the presence of a bacterial pathogen has not been verified*
- *The dangers of antibiotic overprescription are widely known and documented; the availability of more precise, rapid tests, will help to reduce this growing problem*
- *A “gene signature” of 130 genes, detectable by a blood test, has revealed different patterns depending on the type of infection a patient has*
- *Currently, the test requires a full vial of blood and takes a day to process, but development toward a rapid, low-volume, point-of-care test is progressing quickly*

question – does the patient have an infection at all? – can be quite difficult to answer. But once the presence of a pathogen is established, things get even more complicated when that typical question arises: is it a bacterium or a virus?

Viral infections, for the most part, don’t require medical intervention. Bacterial infections will sometimes resolve without intervention, too, but most physicians prefer to prescribe antibiotics if a bacterial pathogen can be confirmed. That “if” is key, though; in the interests of patient health and antibiotic stewardship, it’s important to prescribe drugs only where warranted – and that’s why distinguishing between these various causes of illness is so important. It ensures we get the right treatments to the right patients, and gives us the ability to offer not just a diagnosis, but a prognosis as well. Failing to give antibiotics to patients with bacterial infections could result in their condition declining, rather than improving. On the other hand, giving antibiotics to someone who doesn’t need them exposes that individual to the ever-growing list of drug side effects and also increases the risk of selecting for antibiotic-resistant pathogens. Distinguishing bacterial from viral infections, then, has serious implications for the future health of both the individual patient and the general population.

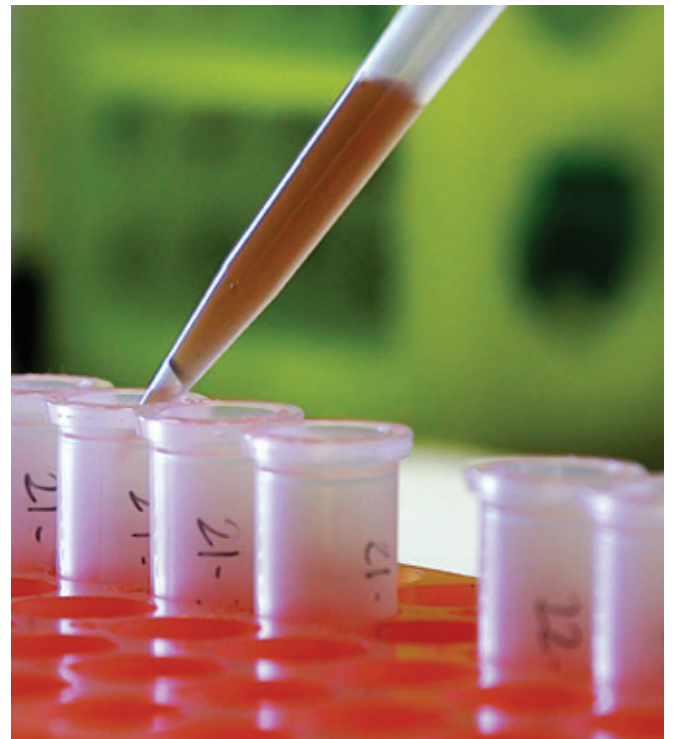
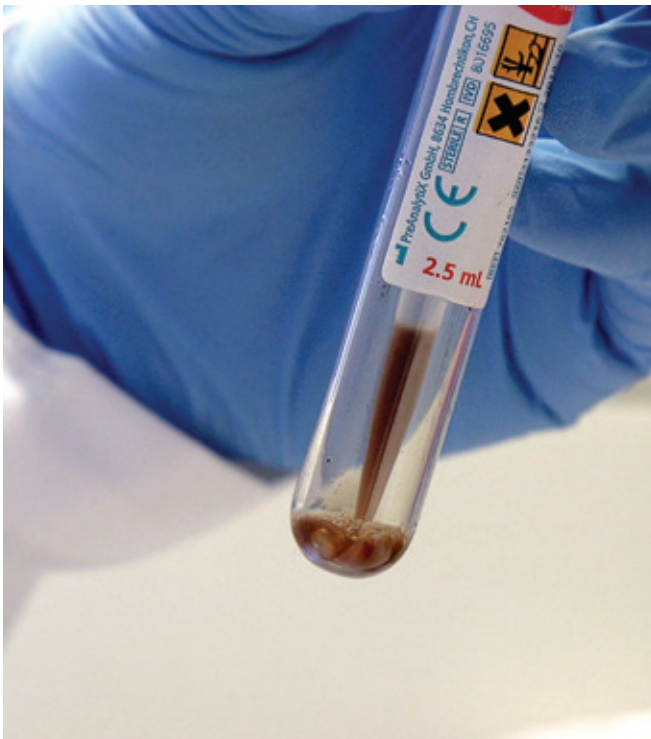
Studying signatures

The human genome contains an estimated 20,000 protein-coding genes, representing the blueprint for everything our bodies need to grow and survive. Some of those genes are active all the time, while others are activated only under certain conditions. Out of that very large number, my colleagues and I have identified 130 genes that are active in a limited set of situations – which include viral infections, bacterial infections, and non-infectious illnesses.

“Distinguishing bacterial from viral infections, then, has serious implications for the future health of both the individual patient and the general population.”

But not all of those genes are active in every case. Some are turned on only when a patient has a viral infection, whereas others are activated only in the case of a bacterial infection, and still others only during non-infectious illnesses. The test we’re developing works by looking at how active each of these 130 genes is in a patient with respiratory tract symptoms. We then compare that patient’s gene activity pattern to the known patterns we describe in our recent paper (1). How well the patterns match determines the patient’s diagnosis.

The specific genes in the signatures were selected to maximize our ability to distinguish between the different illness groups – not chosen because of any known role in the viral or bacterial response. That’s one of the reasons we think it worked so well. We didn’t bias the process by restricting the genes only to those we thought might work; instead, we let mathematical models make those determinations for us. When the genes in the signatures had been selected, though, we did note that most of them have known roles in the immune





App

“Knowing when to prescribe antibiotics is a major challenge in patient care, particularly when managing respiratory infections.”

response. For example, the bacterial classifier includes genes involved in processes like cell cycle regulation, cell growth, and differentiation, while the viral classifier includes ones involved in interferon response, T cell signaling, and RNA processing.

Developing diagnostics

In its current form, the new test is most suitable for research purposes. Why? It requires an entire vial of blood, which then has to undergo a fair amount of preparation. In total, it takes a full day's work in the hands of an experienced laboratory technician – a requirement that we recognize is untenable for routine clinical use. That's why we're working with diagnostics developers to generate a test that would use no more than a few drops of blood, require minimal or even no pre-processing, and return results in an hour or less. Just load the blood sample onto the test cartridge and let it run! Although we haven't finalized a test like that yet, we're making exciting progress toward that goal.

The main steps we need to take now are to put the assay on a testing platform that can be used at the point of care,

and to continue working to show that this paradigm – the host response – can be used in all populations, including infants, the elderly, and all ethnic groups. At the same time, we are expanding the test's repertoire to include not only viral and bacterial pathogens, but also fungal infections. Ultimately, we'd like the test to address both patients in their general practitioners' offices and the challenges of critically ill patients on hospital wards and in intensive care.

Knowing when to prescribe antibiotics is a major challenge in patient care, particularly when managing respiratory infections. Today, without accurate information on the cause of infection, most doctors prescribe antibiotics to ensure they are treating the most dangerous potential cause of infection. But many – perhaps most – respiratory infections are caused by viruses, which means that these drugs are being significantly overprescribed. Our technology is aimed at providing information to help doctors make the best possible decisions regarding which patients truly need antibiotics and which do not. If we can reduce the overuse of unnecessary drugs, then we might see a corresponding reduction in pathogens' resistance. The ideal scenario, should this test ultimately be approved for broad use, is that a patient with a respiratory issue would go to the doctor's office, have a simple blood test administered, and receive results by the time they meet with their physician. Hopefully, that's what we'll see in the not-too-distant future!

Ephraim Tsalik is Assistant Professor of Medicine, specializing in infectious diseases, at the Duke University School of Medicine, Durham, USA.

Reference

1. EL Tsalik et al., “Host gene expression classifiers diagnose acute respiratory illness etiology”, *Sci Transl Med*, 8, 322ra11 (2016). PMID: 26791949.

The Pathologist iPad edition is available for free on the Apple App Store and offers a rich and engaging multimedia experience in a stylish, purpose-built app. The ability to read content online and download the past issue archive make it a must-have for frequent flyers.

<https://thepathologist.com/app>



iPad, iPhone and iPod touch are trademarks of Apple Inc.

Check out our social channels

thepathologistmag 

@pathologistmag 

The Pathologist 

thepathologistmag 

Motion Pictures

A new imaging technique that combines high-resolution pictures with motion information may improve radiotherapy planning for lung cancer

By Soo Kng Teo

When trying to gain an advantage over cancer, the more we know, the better. That's why there's always demand for better, more detailed imaging techniques that provide more information about what's going on inside the patient's body. But sometimes, simply increasing the resolution of the picture isn't enough. Lung cancer is one such situation – even during imaging, patients have to breathe, and the motion of the lungs creates problems. What can we do to overcome the issues inherent in taking photographs of a moving organ? We first need to understand the limitations of what we already have.

At a Glance

- *Increased resolution isn't always the answer when imaging cancer – especially when complicating factors like patient movement are involved*
- *Doctors imaging lung tumors don't currently have an option that provides enough information for ideal radiotherapy planning*
- *A new technique, MRI+CT fusion, can provide continuous motion tracking along with enough detail for treatment planning*
- *To get the new method into clinics, integration issues will need to be addressed and buy-in sought from equipment manufacturers*

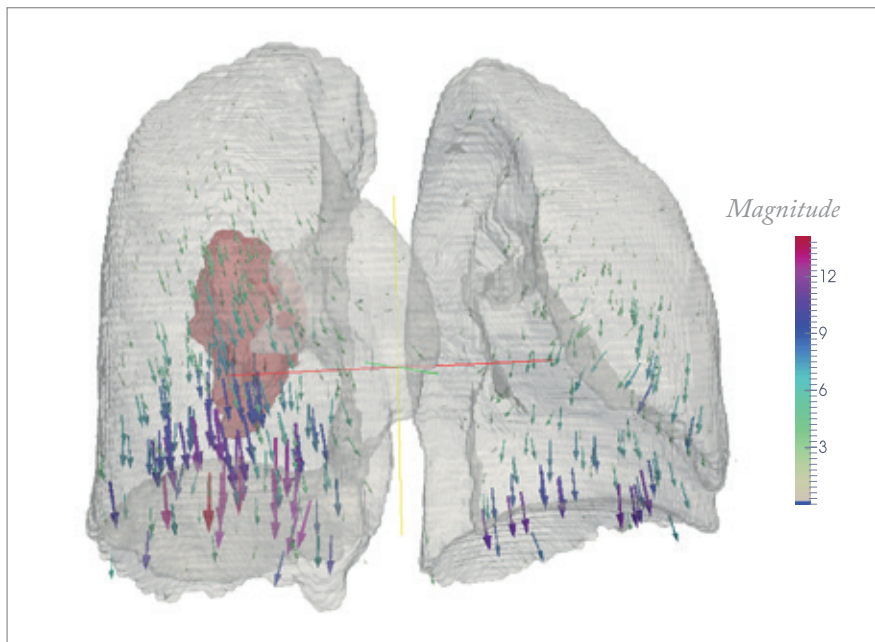


Figure 1. A reconstructed 3D model of a patient's lung from a fused dataset.

The image shows the lung tumor and associated respiration-induced motion (denoted by the colored arrows).

The magnitude of the motion (in mm) is proportional to the size of the arrows.

Lung limitations

At the moment, treatment planning for lung cancer radiotherapy is performed using either static three-dimensional computed tomography (3D-CT) or moving four-dimensional (4D-CT) datasets that track the lungs continuously over time. The main objective of this pre-treatment phase is to plan the patient's radiation therapy so that we can maximize dosage delivery to the tumor while minimizing dosage to the surrounding healthy lung tissue. But that planning is confounded by the patient's need to breathe. That makes the tumor a "moving target" for radiation – meaning that static 3D-CT images just aren't good enough, because the lack of information on respiration-induced motion could result in errors during treatment. Even using a 4D-CT dataset for planning has limitations because the technique doesn't image continuous motion over a single respiration cycle; rather, it captures static snapshots at various points over multiple

respiration cycles and combines them to yield information on an "average" respiration cycle. If the person being imaged can't maintain a regular breathing pattern during image acquisition – not an uncommon problem in cancer patients – then the resulting motion information can be noisy and inaccurate.

Magnetic resonance imaging (MRI) could offer a reasonable solution, as it allows the image capture of continuous lung and tumor motion over multiple respiration cycles, thus overcoming the limitations of 4D-CT. So why don't we use it regularly? Unfortunately, MRI doesn't provide the photon density information required for radiotherapy planning – and their spatial resolution is significantly lower than in CT images. The interior of the lung appears black in MRI images and the airways within the lung can't be seen clearly. These disadvantages haven't made us give up on MRI techniques altogether, but their use in lung cancer radiotherapy planning still needs more research.

Inspired by cross-disciplinary discussions our work is actually the result of a collaborative project with a bioengineer, Poh Chueh Loo from Nanyang Technological University, and two clinical collaborators, Tan Cher Heng from Tan Tock Seng Hospital and Ivan Tham from the National University Cancer Institute. All three are interested in studying the effects of lung and tumor motion on radiotherapy treatment so, working together with them, we developed a new way of examining the lung. Our method allows us to study the motion of the lung and its interior structures – like airways or a tumor – in a continuous manner during breathing. Under the current standard of care, which involves using a single medical imaging modality, we can capture either the lung motion or the interior structures, but not both. So how do we do it? We fuse two modalities into a single technique to obtain all of the information at once.

For our technically inclined readers, the first imaging method we use is 4D-MRI, which lets us continuously track lung motion, but doesn't provide any details about the lung interior. The second method is 3D-CT, which provides a high-resolution view of the internal lung structures, but only at a single point in time. What's unique about our method? We use what we've termed "MRI+CT fusion" to mathematically combine the details in both sets of images, revealing information not visible using the current standard techniques (see Figure 1).

At the moment, radiotherapy planning requires the use of CT images; it can't be done using MRI data alone. We hope that our MRI+CT fusion method will make it possible to use the motion information from MRI for treatment planning – and perhaps one day even replace standard CT scanning altogether, reducing patients' radiation

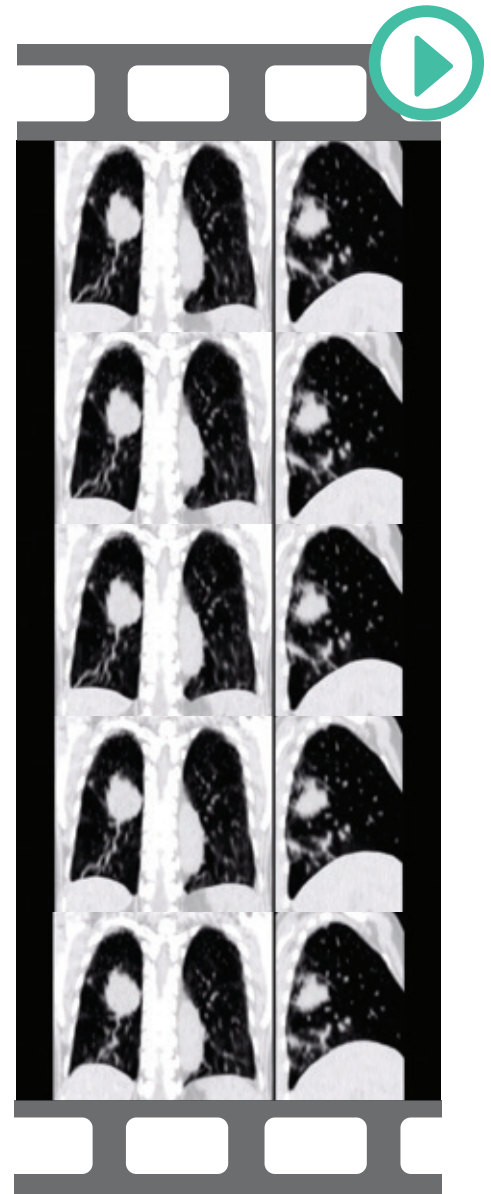
exposure and allowing tests to be repeated as often as necessary.

In the clinic?

Despite the clear benefits, it's still a challenge to integrate our method into the existing radiotherapy clinical workflow – mainly because we foresee integration issues with the hardware and software hospitals currently use. The good news, though, is that these obstacles don't seem to be dampening the clinicians' enthusiasm. We've spoken to doctors working not just on the lung, but in other areas where this kind of information fusion will be useful – and it's encouraging to see that they're trying to apply our method to their clinical problems as well.

When we first started our research, our target audiences were the clinicians and radiation oncologists who specialized in treating cancer with radiotherapy – especially those who focused on lung cancer. But we soon realized that, without access to the hardware and software used in hospitals, it would be very difficult for us to convince the clinicians of the utility of MRI+CT fusion. So at this point, I'd say that the biggest obstacle to the application of our method is getting buy-in from the equipment manufacturers. Without their support, it will be very challenging to integrate our method into the clinical workflow. There are, of course, also a number of regulatory hurdles we have to clear before MRI+CT fusion can even be applied in a clinical setting.

Right now, we are in the midst of exploring new application domains. Our first study focused on the lungs and how their motion affects tumor imaging, but MRI+CT fusion can be extended to studying other organs, too. We're talking to clinicians about other problems where combining information from two – or even more – imaging techniques might be useful. Hopefully, we'll soon be able to expand to other applications. It would



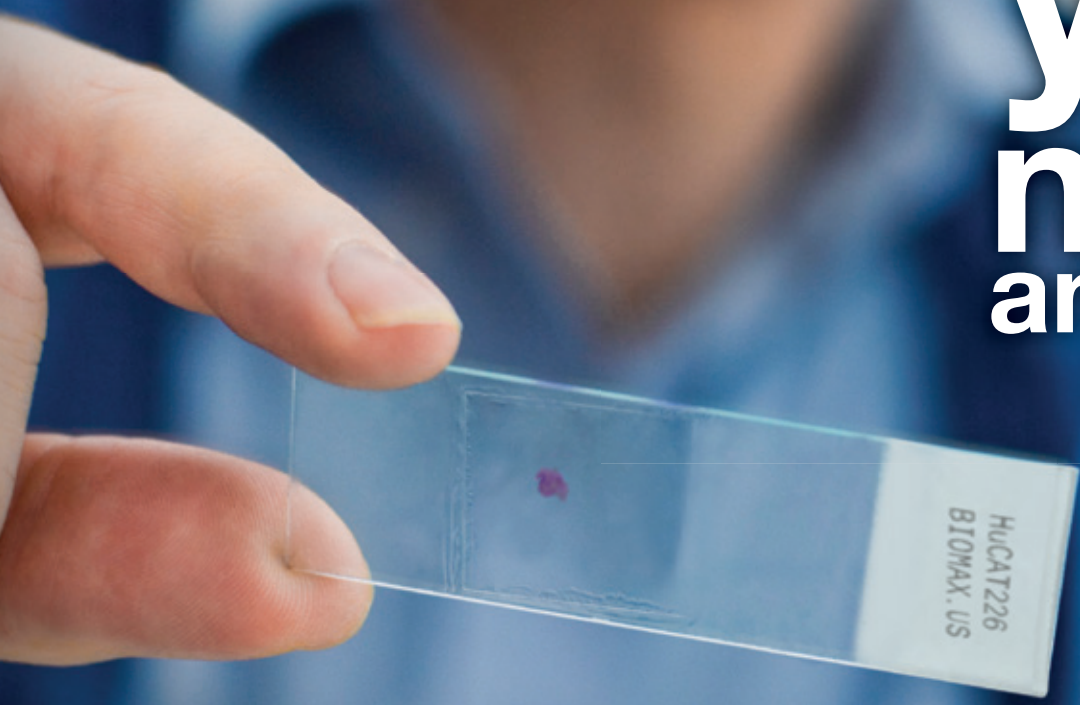
Video stills of the MRI+CT fusion method; the motion of the lung and its interior structures are studied in a continuous manner during breathing, using 4D-MRI and 3D-CT.

be nice to see our method becoming commonplace in the clinic and changing outcomes for patients!

*Soo Kng Teo is a scientist in the Geometrical Modelling department at the A*STAR Institute of High Performance Computing, Singapore.*

ion torrent

Because
you
need
answers



Ion Torrent NGS complete workflow with OncoPrint assays and reporting solutions

When it comes to oncology research, every sample must count. With as little as 10 ng of nucleic acid starting material per pool, you can analyze up to 52 cancer driver genes with the Ion Torrent™ OncoPrint™ Focus Assay, and up to 143 cancer driver genes with the OncoPrint™ Comprehensive Assay. From SNVs to CNVs, indels to gene fusions, OncoPrint assays enable simultaneous detection of multiple variant types from both DNA and RNA.

Coupled with the Ion Torrent™ OncoPrint™ Knowledgebase Reporter, you can confidently analyze results and link them to published data on drug labels, open clinical trials, and scientific guidelines.



Get the answers you need with a complete end-to-end solution. Learn more at thermofisher.com/oncoPrint-assays

ThermoFisher
SCIENTIFIC

For Research Use Only. Not for use in diagnostic procedures. © 2016 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. COL01872 0516

Profession

*Your career
Your business
Your life*



46-49

Science Searches Simplified
Elizabeth Iorns tells us the story behind Science Exchange, the projects her collaboration platform has inspired, and the intriguing ways new research technologies can be used.

Science Searches Simplified

Researcher and entrepreneur explains how she hopes to help others take a great scientific idea to reality

*Michael Schubert interviews
Elizabeth Iorns*

The world of biomedical research is filled with technological possibilities – and the universe of options is constantly expanding. With so many different ways of tackling once-impossible problems, how can researchers keep up? It's clear that no one person can learn all of the techniques needed to bring a project from idea to completion, but collaboration and outsourcing are difficult; it's hard to know who's best-placed to perform a particular experiment, or which service providers are most reliable. When Elizabeth Iorns began her academic career, she faced those same difficulties in her own experiments.

At a Glance

- *As biomedical research becomes more specialized, no single laboratory can perform all of the necessary tests – but the search for collaborators presents problems of its own*
- *Feeling those same frustrations led scientist Elizabeth Iorns to create Science Exchange, an online outsourcing marketplace for researchers seeking problem-free collaborations*
- *Entrepreneurship isn't easy, but research skills are transferable and the number of scientists starting businesses is increasing*
- *For those looking to get into business, the key is to follow your passions – even when it seems risky*



That's what led her to establish Science Exchange, an online marketplace where researchers can locate, evaluate and order from service providers – no muss, no fuss. Here, she speaks candidly about the challenges that entrepreneurial researchers face today, and how she hopes that her online platform might provide the step-up that they need to take ideas to reality.

How has research changed since you began your career?

I think it's become a lot more specialized. As a result, the rate at which scientists are having to learn new skills or purchase new instrumentation has accelerated, and this has created the need for a team-based approach where scientists collaborate more than ever to make discoveries happen. Next generation sequencing is a good example; most scientists aren't trained in that technology, but a lot of them would like to access it. More and more, to conduct their experiments, they're turning to commercial service providers or core facilities with the latest technologies.

A lot of this is being driven by recent developments in digital and molecular technologies – in next generation

sequencing, of course, but also in other fields like microscopy. As new instrumentation and techniques arise, scientists have to increase the depth of their understanding, but that can cost them in terms of breadth. I feel that's a large part of the reason so many researchers are looking for quicker, easier ways of accessing new technologies.

Historically, researchers have focused on learning and doing as much as possible themselves. Nowadays, though, I think we reach a tipping point where we realize that, to be competitive and make big discoveries, we need access to more techniques than we can learn by ourselves. In my opinion, the “modern scientist” has evolved beyond trying to stand completely alone and, instead, focuses on efficiently identifying collaborators who can take on different parts of a project.

What are the major challenges facing researchers today?

Incentives and ownership. Intellectual property and confidentiality are big issues that researchers have to address when they're working with external collaborators. At the moment, those kinds of arrangements are often made on an ad

hoc basis with friends or colleagues, but that can raise issues when expectations and incentives aren't clearly defined. It comes down to the "holy grail" of first authorship. The current system of research incentives prizes that above all else, so in a collaboration, the person who isn't going to be first author on the paper won't prioritize the work as highly as their own – and that leads to conflict. It may be a flawed system, but unfortunately, that's how things work at the moment. That's why I think we need to move toward professionalizing collaboration through the use of contract research facilities.

In some ways, it's a lot easier in industry because ownership is very clearly defined and there are a lot of control mechanisms in place. Pharma researchers know that

they can't just ask someone to collaborate on a project; there always has to be a contract when research is externalized. The protocols have to be clear-cut because industry is very much focused on the end goal – "how do we get the drug to market as quickly as possible?" Conflicts over property and priorities slow the process down, and that's not acceptable.

In academia, though, there's still a strong focus on individual contributions and "personal branding." I hope that will change soon, because it doesn't reflect the reality of modern science – that everything is based on teamwork. If you look at any Nature or Science paper now, it's likely to have more than 10 authors. There's got to be a way to credit people who aren't necessarily first authors, but

who have contributed to a lot of different research projects. Those researchers are the backbone of progress, and we need to ensure that they also have good career opportunities.

What is Science Exchange?

It's a marketplace for outsourced research services. We have a network of more than 3,000 service providers, including contract research organizations, government facilities, and academic laboratories. We've put in place fee-for-service agreements with all of our suppliers so that any researcher, whether industry or academic, can use our marketplace to order an experiment with any of those service providers under our pre-existing contracts. That provides two benefits: one, efficiency,

Grand Plans

Science Exchange's Reproducibility Initiative and Elizabeth Iorns' ambitions to produce the largest public dataset of reproduced work in the field of biomedical science

The Reproducibility Initiative aims to provide an efficient way to run replication studies. Frequently, pharmaceutical companies and venture capitalists would bring us published studies and ask us to re-run the results. Just as we began to wonder why they were doing that, two publications came out that indicated that only about 20 to 25 percent of studies could be reproduced. So we decided that, although we were happy to provide private validations as a service, we also wanted the ability to publish those results so that the information was

freely available. After all, what happens if a private validation fails – but it's never published, so no one benefits from it?

From that starting point, we reached out to more than 20,000 recently published authors to ask if they would be willing to have their studies reproduced. About 2,000 of them agreed, so we used that as the basis for a funding application – which taught us that, although funders were very interested in replication studies, they thought that the opt-in model would be biased toward reproducibility. So instead, they funded us to do the Reproducibility Project for Cancer, which was based on a selection of 50 high-impact cancer biology studies.

That project is a collaboration between Science Exchange and the Center for Open Science. It uses the Science Exchange network and the Center for Open Science framework to replicate the experiments from these 50 high-impact cancer biology studies. The goal is to produce the largest public

dataset of reproduced work in the field of biomedical science. The project is about halfway through now, so we're just starting to get all of the results back. We'll publish them in batches as they come in, and at the end, we'll run a meta-analysis of factors associated with reproducibility.

Since we began the project, we've had a lot of interest in validation studies from other groups. I'm optimistic that, as people see the results come out, they're going to be more interested in funding replication studies in their own fields of interest. Most research funding is allocated to original work, but we're hoping to provide a structured path to fund validation studies, because they're vital to clinical translation. It's problematic that industries currently run these studies independently, because they don't publish the results, so the work is repeated and resources are wasted. That's what we're hoping to overcome with the Reproducibility Initiative.



Commitment to Conservation

Elizabeth Iorns describes why she feels the Genetic Diversity Project can really make a difference

The Genetic Diversity Project is a really cool undertaking that raises awareness of both endangered species and the unique ways people use technology for conservation. The Kākāpō 125 Project, for instance, leverages the Science Exchange network to sequence the genomes of every remaining kākāpō, a critically endangered species of New Zealand flightless parrot. Why? It lets the Kākāpō Recovery Team look for the most effective ways to match the birds in breeding programs – and it’s already showing significant success. This past year was their most successful yet in terms of chicks born. The number “125” used to indicate the number of known living kākāpō – but it’s probably not accurate anymore!

People keep finding newer and cooler ways of using genome sequencing. There are conservation initiatives out there like the Kākāpō 125 Project, and there are investigations into the vagaries of the human genome – like the Resilience Project, which looks for protective mutations against human genetic diseases. People used to hear “genome sequencing” and think “tumor biology,” but that’s no longer the case – and I think that’s fantastic.

because they don’t have to go through the contracting process with each individual supplier they need; and two, it aligns incentives. Ownership is clearly defined in advance, and the incentive is simple: payment for a body of work. But by consolidating spending and handling all of the logistics – supplier contracts, payment processing, compliance with regulations – we offer not just trustworthiness and ownership guidelines, but also efficiency.

At the moment, Science Exchange offers more than 5,000 different experimental services. The vast majority of our work is focused on biomedical research, so examples include safety pharmacology, xenograft models, efficacy testing, and access to new technologies like CRISPR/Cas9, single-cell sequencing and next generation sequencing. But we also have other experimental services, including chemistry, materials science and more. Even NASA has used Science Exchange to develop the blackest material that was ever measured!

What inspired you to create such a service? Before I started Science Exchange, I was an assistant professor at a time when the research ecosystem was changing significantly. There was an increasing need for easy access to a broad range of technical service providers. When I moved to the University of Miami, I was trying to continue my work with microarray technology, which – while now very old-school – was cutting-edge at the time. I wasn’t in a position to set up collaborations, so it was really difficult to know where I could turn to get those experiments run.

Looking for commercial service providers outside our institution involved a lot of contracting, set-up paperwork, payment arrangements, and uncertainty, because I had no way of being sure they’d do a good job. That’s when my co-founder, Dan Knox, and I came up with the idea that you could solve a lot of those problems with an online marketplace that provided

transactional histories and removed the issues with contracting and payment.

Of course, every scientist wants the ability to control every single detail of an experiment, and outsourcing prevents that. You have to have a lot of trust in your service providers. Unfortunately, many of them have high staff turnover rates, which can make it difficult to keep up with which ones are best at any given experimental service. We found that word of mouth tended to fall out of date very quickly. Our solution to that was the “supplier scorecard” – essentially a user review, so that people can see others’ experiences with service providers. After every contracted experiment, we ask the requester to fill out a scorecard. That’s given us a lot of insight into the real-time performance of our service providers.

In February 2011, we applied to the Silicon Valley accelerator program Y Combinator to get Science Exchange off the ground. During the three months of the program, we were able to build and launch the initial minimal viable product – and, surprisingly, generated a significant amount of growth. Researchers found out about Science Exchange and wanted to use it. That helped us raise additional funding to build our team, expand our supplier base to offer more services, and improve our marketplace website (www.scienceexchange.com). Now that Science Exchange has really taken off, we’re focusing on growth through digital marketing, word of mouth, and institutional deals.

Who uses Science Exchange, and how?

Most of our volume comes from large pharmaceutical companies and well-funded, early-stage biotech companies. These are organizations that have money, but need speed; they want to be quick to market, so they’re trying to drive their research forward as quickly as possible. For them, Science Exchange is an ideal solution, because they can access all of

the service providers without any delays for contractual issues. They can even push their research in parallel through multiple providers to avoid reductions in speed.

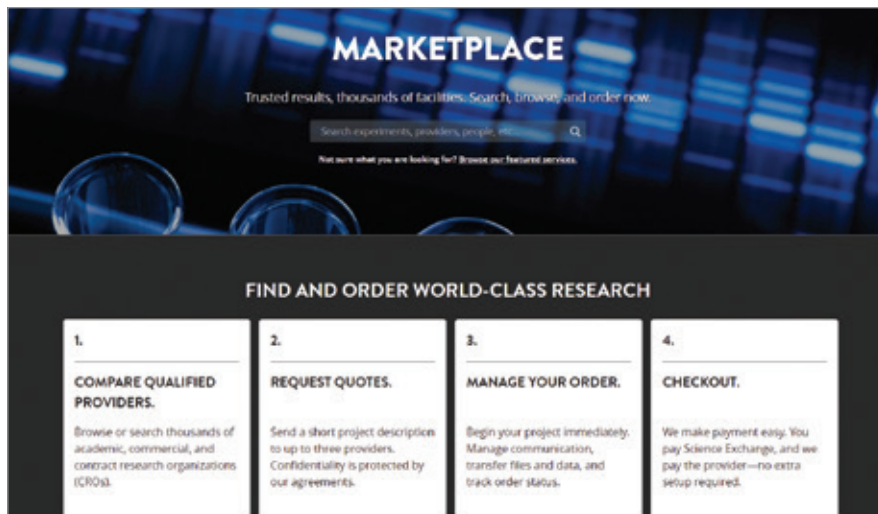
Using Science Exchange is very straightforward. Requesters simply log in, search for a service, request quotes, and place their orders. The transactions are automatically covered by supplier agreements regarding intellectual property and confidentiality. If a user searches for an experiment and finds that there are no current providers, they'll be brought to our sourcing service, which looks through a much larger database. They can then choose from the available options and we'll add their preferred provider to the Science Exchange network.

For those wanting to become suppliers, applications are screened in under a week, and if the supplier passes, they're "onboarded" into the Science Exchange network and trained to use the platform. Not everyone is accepted immediately, though. It's a balancing act – we want to have all of the suppliers we need, but at the same time, if we add too many, they won't each get enough work. We selectively add suppliers when our requesters have a real need for their services.

What's day-to-day life like for a science entrepreneur?

It's not that different to being a scientist. One of the things I like to emphasize is how many of the skills that you develop in science are applicable to entrepreneurship – obtaining funding, giving presentations, interpreting experimental results and deciding what to do next... You think of developing a company as a research project where you test out different theories about what's going to help your company grow, and then you look at the results, see what did and didn't work, and build from that.

Individual investigators in academia have a lot of freedom to make their own decisions about their research, look for funding opportunities, and drive projects



forward. In many ways, I think business is pretty similar. Academia has certain frustrating areas of bureaucracy that you don't have as an entrepreneur, because you get to decide how much bureaucracy there is! But business comes with its own challenges; you have to worry about stability, and you're responsible for many more people's careers and livelihoods as you make sure that the company is sufficiently funded to support its staff.

What's your advice for other scientific entrepreneurs?

We're seeing a rise in entrepreneurship. More and more scientists are trying to bring new technologies and services to the market, which is really exciting. I've also noticed significant strides in terms of people's awareness of the opportunities, and that's meant that a more diverse group of people are starting new companies – especially in Silicon Valley, where there have previously been fewer women leading businesses. I think that's great, because that's really going to drive innovation.

To launch a new business, I think it's important to leverage infrastructure that already exists; you don't want to spend time and resources reinventing the wheel. Science Exchange gained a lot by taking

part in Y Combinator, and I would strongly advise other entrepreneurs to look for similar opportunities. Instead of thinking about problems that have already been solved, you want to focus on building your company. So if you can go somewhere where they can teach you the routine steps of setting up a business, you have more time to focus on the unique parts of your project.

In general, you should look for the things that passionately interest you. A lot of people thought I was crazy for leaving my job to start Science Exchange, but I really felt like I could make more of an impact with that than I could as an individual investigator. And within the first year, I knew I had made the right decision. Even if Science Exchange had failed, I still felt that my personal and professional growth were accelerated because I was doing something I was really passionate about, rather than just sticking to the safe path. People should look for the areas that most excite them, because even if that seems risky, the rewards will be worth it.

Elizabeth Iorns is co-founder and CEO of Science Exchange, Palo Alto, USA. She is also a part-time partner of Y Combinator (Mountain View, USA), and mentors at IndieBio (San Francisco, USA).

A close-up portrait of Jennifer Grandis, a woman with short, wavy, grey hair, smiling warmly. She is wearing a grey and white striped top and a necklace with large silver links. The background is a soft, out-of-focus light blue.

Perfecting the Translational Balancing Act

Sitting Down With... Jennifer Grandis,
Professor, Department of Otolaryngology – Head and Neck Surgery,
Associate Vice Chancellor – Clinical and Translational Research,
University of California, San Francisco, USA.

You've chosen a dual career as both clinician and researcher – why?

Primarily, I was motivated by the problems that confronted my head and neck cancer patients when I was in residency. I was being taught how to surgically excise their tumors but, more often than not, such patients still died of cancer, and the surgery had profound consequences on their appearance and their ability to speak, swallow, taste and interact with the world. I found that very unsatisfying, and as it was not a cancer that was commonly studied by biologists, I felt that if I was really going to make a difference to these people's lives, I needed to learn about cancer biology.

Having a clinical practice as well as doing research has been a really comforting balance because research requires coping with disappointment and rejection, while taking care of people is immediately gratifying.

What drives your research?

I would call it precision medicine. We're trying to elucidate the most effective targets for each patient or patient subgroup. Historically, we've treated cancer as one disease, and either cut it out or given radiation or chemotherapy that indiscriminately kills dividing cells. That is not effective in a lot of cases, and particularly in head and neck cancer. Now, we have elucidated the genomic basis of the disease and discovered that instead of being one cancer, it is dozens of different cancers, each slightly different. We're diving deep into these subtypes to identify the features that are critical for propagation, with a view to developing treatments. The last drug approval by the FDA for head and neck cancer was in 2006, so we really need new treatment options.

You mentioned that research can be very frustrating; how do you cope?

First and foremost, having a sense

of humor and not taking myself too seriously! It helps to have a variety of projects percolating so that if one reaches a dead end there are other ways that I can continue to be productive. And I keep my eyes on the prize: translating our work to help people. I spend a lot of time training graduate students, postdoctoral researchers and junior faculty, so it's my job to model how to deal with rejection and stress.

How do you feel about mentoring the next generation of researchers?

It's the best part of my job. It's not about me, it's about inspiring others, more talented than I, to choose this life. It's not an easy choice these days. I don't think I even knew what an NIH funding level was when I was starting out. It just didn't occur to me that outside factors could influence or curtail my ability to do what I wanted to do. But the combination of the shrinking federal budget in the United States, the paucity of tenure-track positions and the lack of resources of most academic medical centers means that the life of an independent investigator is no longer held out as a feasible goal for many trainees. I feel it's my job to show people that a career in research is still possible and rewarding.

Any advice for other mentors?

You have to be a good listener and tailor your mentoring to the needs of the individual. One size does not fit all, so you can't take a dogmatic approach. It's also important to understand that mentoring doesn't just happen – you have to sit down and dedicate the time to it. Finally, you must be able to disentangle your needs from those of the mentee. So often in the world of science, mentors are at the stage in their career where they feel they need to own a project and claim a certain amount of credit in the form of authorship order, and so

on. It's an inherent conflict and I think you have to call it out and figure out a way to solve it.

You started your role as Associate Vice Chancellor at UCSF almost a year ago – how did you approach it?

I feel the administration in an academic medical center should do two things. First, it should get out of the way – reduce the administrative burden and allow investigators to be creative and engage with science. Second, when investigators need support, the university needs to be there to facilitate – particularly when it comes to clinical trials, which no one, however talented, can do alone.

As soon as I started, I had to begin work on renewing our Clinical Translational Science Award. Taking on a leadership role, learning about the institution and simultaneously putting a very large grant application together was a real baptism of fire!

What's next on the agenda?

Now, we need to actually do the work we proposed! One project that I think will be very gratifying is developing a centralized solution for biobanking at UCSF. We have over 150 legacy biobanks at the university and my goal is to provide a coordinated infrastructure so we have a transparent library of bio-specimens. People can choose to share specimens or not, but everybody in the institution – and frankly in the state of California and the world – deserves to know what we've collected. That kind of information can really stimulate collaborations and drive research. My own work has always been very dependent on bio-specimen acquisition and I'm convinced that a unified approach will help everybody.

First published in The Translational Scientist (www.thetranslationalscientist.com), a sister publication of The Pathologist.

Your Complete Aperio Pathology Imaging Solution for Fluorescence Research

Get the most from your fluorescence samples – without fear of fading!

Leica Biosystems has the complete solution to scan, manage, and analyze multi-channel Immunofluorescence and FISH slides with ease:

- Aperio VERSA* is a flexible, brightfield, fluorescent and FISH scanner, creating crisp, high-quality images.
- Aperio Image Analysis tools provide comprehensive, quantitative data, eliminating time-consuming manual review.
- Aperio Image Analysis Workstation enables you to manage your slides and data locally.
- Aperio eSlide Manager offers remote access, sharing and collaboration.



**Choose
Aperio Digital Pathology
for a solution tailored to
your research needs.**

* Aperio VERSA is for Research Use Only. Not for use in diagnostic procedures.

Certain products are CE marked and labeled for specific clinical applications. Unless otherwise labeled, all other products are intended to be made commercially available "For Research Use Only. Not for use in diagnostic procedures."

Copyright © 2016 Leica Biosystems Imaging, Inc.
All Rights Reserved. LEICA and the Leica logo are registered trademarks of Leica Microsystems IR GmbH. Aperio is a registered trademark of Leica Biosystems Imaging, Inc. in the USA and other countries.