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What's got you talking on our website this month?
www.thepathologist.com

Lab Turnaround Time Study Sets Alarm Bells Ringing:
thepathologist.com/issues/0114/503

"On Time Deliveries - the only measurement that counts

During my years with the College of American Pathologists Quality Practices Committee, I either wrote or in some way contributed to most of the TAT QProbes studies we published. I am embarrassed to say that never once did we confirm that faster TAT improved care or affected outcomes. I now believe that TAT is the wrong measurement to track.

Certainly, it is in pathologists' interests to reduce TAT and its companion, throughput: slide boxes left untouched today will only accrue work hours tomorrow. But why should our customers want to concern themselves with our operational efficiency? I suspect they care only about outcomes – not how long it takes us to turn out our reports, but only whether or not they have those reports in hand when they need to make treatment decisions. We may be beating ourselves up to collect measurements that our customers find meaningless."

Perhaps it is time we adopted the standard metric by which all other industries gauge timeliness of service, one for which their customers perceive value, namely measurement of 'on time deliveries (OTD)'."– David Novis, US

The High Price of Diagnostic Error:
thepathologist.com/issues/0715/302

"Adverse Effects of Reporting Test Results as Fragmented Data

Hello Dr. Plebani,
Are you aware of any studies in the peer-reviewed literature examining the diagnostic error and medical negligence risks produced by EHR, PHR and HIE platforms that are still displaying the cumulative results of clinical lab, imaging and all other available tests to both physicians and patients as difficult to read, incomplete and fragmented data, instead of the comprehensive, integrated and actionable information they really need? In the United States, this problem is a major understudied and unmeasured area of patient safety and unnecessary testing costs." – Bob Coli, US

Last Month's Top Tweets

The Pathologist @pathologistmag Sep 8

Another busy day in Belgrade #ECP2015
10:09 AM - 8 Sep 2015

Michael Misialek, MD @DrMisialek
Know a great pathologist? Nominate them to the Power List *https://thepathologist.com/#issues/0615/its-time-to-cast-your-vote-the-pathologist-power-list/…* @pathologistmag @Pathologists @TheUSCAP @ASCP_Chicago
3:57 PM - 4 Sep 2015

Einstein Pathology @EinsteinPath
Thx @pathologistmag for article & quote calling for reversal of NYS bill banning #pathologist - patient interaction.
5:52 PM - 10 Sep 2015

The Pathologist @pathologistmag Sep 7
#Pathology education is critical. Importance of #diagnostics is only increasing, says Han van Krieken #ECP2015
3:03 PM - 7 Sep 2015

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Collaborate or Crash

To fail to change is to fail patients

At this year's European Congress of Pathology in Belgrade, a senior director of a multinational manufacturing company told me, "It's time for pathologists and manufacturers to club together to inspire change and to ensure that patients get treated earlier." In essence, he recognized the truth that collaboration is essential for true personalized therapy. But collaboration is a broadly used and sometimes nebulous term. What does it really mean? "Working with others to do a task and to achieve shared goals," according to Wikipedia – a definition that is accompanied by an image of a team building a 15-meter-tall human pyramid, a feat which must be particularly perturbing for the man who must climb to form its peak. No less worrying – but I would argue more challenging – are the mountainous challenges that must be overcome by those who operate in the field of diagnostics.

Unarguably, our ever-increasing knowledge of genetics and disease, and the resulting growth of molecular diagnostics and personalized therapeutics is improving healthcare. But what does this expanded knowledge mean for those operating in labs? Higher workloads, continuously revised educational curricula, the necessity for new technology and techniques, growing financial pressures...

And what about those manufacturing the companion diagnostics and developing molecular technologies? The rapid expansion of personalized medicine certainly presents a great deal of commercial opportunity, but not without significant challenges, including the need to develop products that suit small and high volume labs (both in terms of budget and capabilities) – no easy task. And let's not forget the rising financial constraints faced by their potential customer base and the difficulties in convincing purse holders (governments, health service providers, hospitals) of the long-term value of new products and technologies. The overall outcome is a painfully slow uptake of molecular diagnostics. Who is most affected? The patient.

This month, we tell the story of an ambitious collaborative endeavor in the UK that aims to ease the perilous climb, by bringing together pathologists, a companion diagnostics manufacturer, quality assurance service providers and the UK's National Health Service. And though the story is inspiring, a great many more ventures of a similar nature will be needed if the challenges are to be met on a wider scale. Notably, such collaborative efforts demand a change in the mindsets of pathologists and lab professionals.

To quote the head of a Spanish university pathology department who passionately spoke with me on the topic: "As pathologists, we need to step out of our comfort zone. We need to start behaving differently – now."

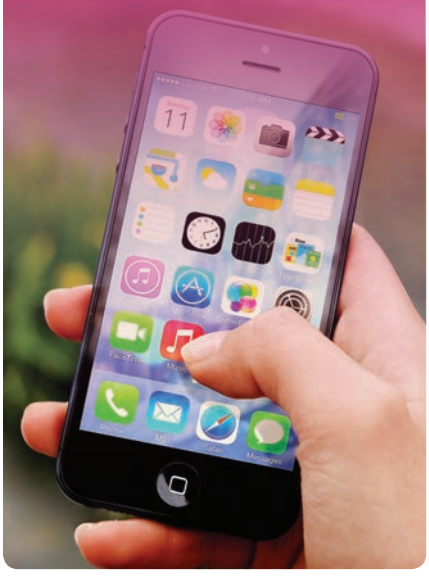
Fedra Pavlou
Editor

Upfront

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

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

Email: fedra.pavlou@texerepublishing.com



The Uberification of Phlebotomy

Need a blood sample? There's an app for that…

By now you've probably heard of (or perhaps used) Uber, the on-demand travel app that allows customers to connect directly to a taxi driver in their area, rather than going through a taxi booking service. With so many conveniences now available directly through our smartphones, why not blood tests? A US company has embraced this so-called "Uberification" and created an on-demand phlebotomy service.

Known as Iggbo, the company works in a similar way to Uber: doctors can order a test using the app, which will then appear to a pool of independent, qualified phlebotomists in the area, who can accept or decline the job. Priority goes to "favorites" selected by the physician and those with the highest rankings. Once the job has been accepted, the phlebotomist will receive instructions on the collection, preparation and shipping of the sample. Patients can also have the option of having their sample taken at a convenient location, such as their home or workplace.

"There are certainly market parallels between Uber and Iggbo," says Nuno Valentine, Iggbo CEO. "In Uber's case, there were previously legions of badged taxi services exclusively entitled to offer per-ride transportation. Uber saw a diverse, geographically-spread community that had a shared need for dependable, high-quality service delivery. We saw similar characteristics in the healthcare community," he explains. "Historically there has been a disconnect between the labor – the phlebotomists who draw a patient's blood – and the demand from physicians who are ordering the tests, making it difficult to coordinate physician orders for lab tests, phlebotomist availability and patient schedules."

What inspired the idea? A US Department of Justice Investigation last year discovered that some labs were paying doctors who sent in test samples. This sparked a US government fraud investigation into the offering of financial incentives to doctors to order – often unnecessary – tests (1). "The government really produced the opportunity," says President and co-founder of Iggbo Mark Van Roekel, "and the government did the right thing, frankly. In the way it was set up before, there was a passive incentive that was in place for physicians to order more tests than they perhaps should because there was money to be had."

For labs, Iggbo offers access to patient samples without the need for large investments in location, infrastructure and staff, which the company hope will allow labs access to more patients. For patients and doctors the service is convenient, and free. "In the US, the current system already sees one in three prescribed blood draws failing to take place – for independent labs, these inefficiencies reduce access to patients," adds Valentine.

In eight months, Iggbo has reportedly attracted 4,000 phlebotomists, and is now operating in over 18 states, with plans to expand further. Does it have the potential to transform sampling in the way apps like Uber changed our approach to taxi hire? Time will tell, but the allure is clear: "Physicians can be confident of patient compliance with necessary lab tests. Patients can have blood drawn at their convenience. Phlebotomists can work when they want, where they want," concludes Shaival Kapadia, Iggbo Chief Medical Officer. *RM*

Reference

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Game-Changer or Media Sensation?

Predictive test shows promise in catching breast cancer relapse, but caution urged over sensationalist headlines

A new blood test is offering hope of identifying early-stage breast cancer patients at risk of relapse after apparently successful treatment, on average, eight months before clinical relapse occurs. Understandably, news of this possible breakthrough has gained an immense amount of public interest, with headlines reporting on a "simple" blood test (1) which can "determine if your cancer will come back" and is set to make biopsy "unnecessary" (2) – but the reality isn't so straightforward. We take a look at the science behind the headlines...

The study, conducted by researchers at The Institute of Cancer Research, London, UK, used personalized digital PCR (dPCR) for circulating tumor DNA (ctDNA) analysis. In a cohort of 55 women with early-stage breast cancer, massively parallel sequencing was used to characterize the mutations present in their individual tumor biopsies, and personalized dPCR assays for each somatic mutation were then used for "mutation tracking" – using serial samples following treatment to identify each patient's specific mutations in their plasma (3). dPCR is a highly sensitive method, which can identify very small amounts of mutant DNA, making it an ideal approach for tracking tiny quantities of tumor DNA in the blood.

The results demonstrated the potential clinical utility of ctDNA analysis in this scenario: 19 percent of the patients had ctDNA present in their plasma just two to four weeks after cancer surgery, and this was found to be a significant predictor of early relapse. For patients who were assessed with "mutation tracking" at six-month intervals, 80 percent of patients had ctDNA present in their blood prior to relapse. In patients who didn't have a recurrence of their cancer during the time of the study, 96 percent did not have ctDNA detected in a postsurgical sample or via mutation tracking. The method appeared to be effective in all breast cancer subtypes, but was particularly sensitive in ER positive cancer – a promising result.

However, there were clear limitations: this was a small study with only two years of follow-up, and there is as yet no information on how many of these patients might go on to relapse at a later date. It appears the test cannot spot brain metastasis – in three patients with cancer restricted to the brain, no ctDNA was detected, potentially because the blood-brain barrier prevented it from entering circulation.

The hope is that the earlier relapse is detected, the earlier treatment can begin, which could mean better outcomes for patients. But the relative expense and highly individualized nature of the test means that getting it to the clinic is unlikely to be quick or easy, and further validation is needed. "Ours is the first study to show that these blood tests could be used to predict relapse. It will be some years before the test could potentially be available in hospitals, but we hope to bring this date closer by conducting much larger clinical trials starting next year," says the trial leader, Nicholas Turner. *RM*

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A New Forensic Body Clock

A novel approach using muscle protein could be used to more accurately predict time of death

Credit: Alan Cleaver

Determining postmortem interval (PMI), the time which has elapsed since a person has died, as exactly as possible is a vital component of many forensic cases. Currently, body temperature is the most accurate method of determining PMI, but depending on the environmental temperature, clothing, age, as well as a number of other factors, this method is only useful for up to 36 hours after death, leaving the door wide open to techniques which can accurately work after this time (1). So it's no surprise that methods for determining PMI are constantly being re-evaluated and refined.

Now, a team headed by Peter Steinbacher at the University of Salzburg, Austria think they might have made a breakthrough. They have identified a series of changes in the presence and activity of a number of proteins present in skeletal muscle which could be used to accurately determine the PMI up to 10 days from the time of death (2). "The breakdown products are present for a specific time – so if you know which of these products are present... then you know when the individual died", explains Steinbacher, whose work built upon pre-existing research into meat tenderness, which demonstrated that a number of muscle proteins reproducibly degenerated into the same products, even across different species (3,4). This degradation of muscle protein is down to a group of calcium-dependent proteolytic enzymes called calpains, which are activated once the integrity of the sarcolemma is compromised, increasing intracellular calcium.

Using skeletal muscle has several benefits when it comes to forensics; it is the most abundant tissue in the body and comes with a greater delay in postmortem change when compared with other tissues in the body, vital for that post 36 hour PMI reading.

Using Western blotting and SDS-PAGE gel electrophoresis, Steinbacher assessed the degradation and appearance of a number of muscle proteins over a period of 240 hours postmortem. The experiments revealed protein changes of varying significance; some proteins, such as titin dp2 appeared in a relatively large time range, whilst others, such as the degradation of titin 1, occurred in a much more limited timeframe. Steinbacher claims that when used in combination, these patterns could be used to effectively characterize certain time points, allowing a more precise determination of PMI.

As a similar protein degradation pattern appears in several other vertebrate species, the research team analyzed over 60 human tissue samples from the university's forensic department where they found related patterns. "Research with human samples is always very difficult, as there is no way to influence any variables or to standardize experimental conditions", says coauthor of the associated study Pittner Stefan. Despite this, Steinbacher remains positive, claiming that once implemented, the accuracy will steadily improve as the database compiling the information expands. The team now intends to continue its work by examining the effect of other variables on pig muscle protein, including temperature, gender, body mass and humidity. *JR*

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Pregnancy Risk Predictor

Will the risk of miscarriage or the promise of twins make it into at-home tests?

"Pregnant", "Not Pregnant": the two simple answers that you would expect all currently available pregnancy tests to provide. Picture this though… a home-based test that could predict likelihood of miscarriage, aneuploidy or even twins. Would putting that kind of information into the hands of consumers be a good thing? And how likely is it that such a test will be developed?

UK-based MAP Diagnostics believe in the value of such a test. In fact, their entire ethos is based on the empowerment of parents by providing rapid, reliable results, early in gestation to allow them to make informed decisions. To that end, it is using protein profiling to develop a test which provides early indications of pregnancy-related problems. Employing a technique more commonly applied to assisted reproduction, the company's urine test will use mass spectrometry to identify protein biomarkers released by embryos, which will then be analyzed using an algorithm developed using a database of pregnant women, to spot patterns linked to various outcomes, such as Down's syndrome (1). Using this approach, the company hopes to develop tests for a range of pregnancy-related conditions including gestational diabetes and ectopic pregnancy, and to predict a number of complications including intrauterine growth restriction and miscarriage.

The approach will need a lot of further validation, but initial results for trisomy prediction look good – using an archive of 101 maternal urine samples, the test had 100 percent sensitivity and specificity at 12–14 weeks gestation. Although cfDNA screening is also being explored for this purpose, the creators of the urine test believe that, as a less invasive alternative which will reportedly be a fraction of the cost, protein profiling could offer a compelling alternative – and they plan for more tests to follow. *RM*

Reference

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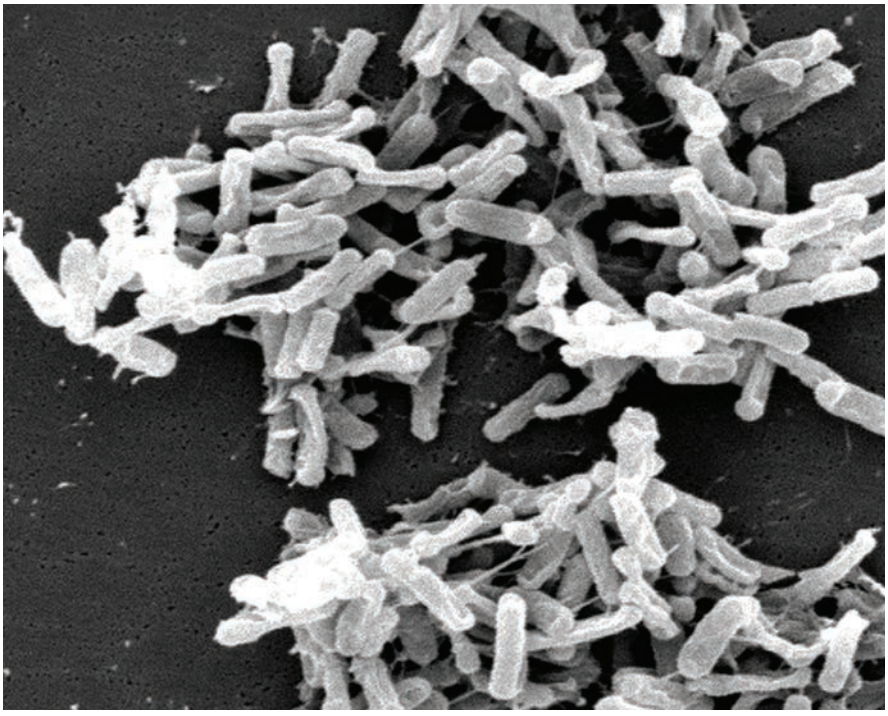
Molecular Testing Makes All the (C.) Diff

Diagnosing Clostridium difficile infection with molecular methods alone could lead to overdiagnosis in around 50 percent of patients



Clostridium difficile infection (CDI) is a significant cause of illness in hospital inpatients; worryingly incidence rates have increased by over 200 percent in the last 15 years alone. Hypervirulent strains are suspected to play a part in this, but could overdiagnosis also be a key factor? A team of US researchers certainly think so, in fact, they believe that up to half of patients diagnosed with CDI using molecular testing alone could be overdiagnosed, leading to unnecessary treatment.

Traditionally, immunoassays to detect *C. diff* toxin were the standard for identifying CDI because they provided faster, more reliable results than bacterial culture. The advent of molecular diagnostics, however, coupled with the FDA approval of the first CDI molecular test in 2009 has led to widespread uptake of the technique for CDI diagnosis in hospitals (1). Though methods like PCR provide fast and sensitive results, there is one major flaw – similar to culture, PCR does not detect the bacterial toxins which correlate with clinical disease, but rather the toxin-producing genes.

Since switching to molecular testing, some hospitals have reported increases in CDI detection of 50 to 100 percent (2). But because they don't detect toxins, are molecular tests detecting disease or simply *C. diff* colonization, and could the increase in CDI incidence be partly attributed to the introduction of these tests rather than a rising incidence? These were the questions researchers at the University of California Davis Medical Center, sought to answer. Using both PCR and toxin they tested 1,416 hospitalized adults with diarrhea and suspected CDI, and assessed duration of diarrhea, complications, and CDI-related deaths. What they found was that toxin-negative, PCR-positive patients had similar health outcomes to patients who returned negative results on both tests, showing less inflammation and milder symptoms even with little or no treatment. This strongly suggested that those patients did not need treatment for CDI, and that their nosocomial diarrhea had another cause (3).

Overall, 55.6 percent of the patients with a positive PCR result had no toxin present using immunoassay, implying that molecular testing alone could overdiagnose as many as one in every two patients. The authors concluded that relying on molecular tests to provide a diagnosis is likely to lead to overdiagnosis, unneeded treatments and increased costs, and recommend that there should be more focus on developing reliable ways to distinguish between active infection and bacterial colonization. The results serve as a reminder that, even as molecular techniques continue to become more commonplace, DNA testing isn't always the best approach. *RM*

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The Osteoporosis Oracle

What do geochemistry, biology and space science have in common? They've all come together to develop a new, real-time test for bone disease

It is estimated that over 200 million people worldwide suffer from osteoporosis (1). The condition, which disproportionately affects adults over the age of 50, is also a concern for astronauts, who shed 1–2 percent of their bone density per month (2). Now, a new test developed by scientists at Arizona State University and The Mayo Clinic, in association with NASA, could monitor the development of asymptomatic bone disease in real-time, earlier than current tests, after being verified by astronauts posted on the international space station (3).

The method, unveiled at the Goldschmidt conference, uses the calcium isotopes, ^{42}Ca and ^{44}Ca, as biomarkers for bone diseases such as osteoporosis and multiple myeloma. The team discovered that these isotopes, which are absorbed from the blood during bone growth and development, are also released back into the blood stream once bones begin to break down. Using mass spectrometry, the researchers measured relative ratios of the isotopes from serum and urine samples to calculate whether bone is being resorbed or formed.

Though the initial study assessed bed-bound patients who are known to experience bone mass loss, the focus moved to the skies for a less controlled population. In collaboration with NASA, the team measured calcium isotope ratios from 30 astronauts before, during and after their missions to the international space station. The results were consistent with those of bed-bound patients, finding an increase in calcium excretion. The study was expanded to 71 patients with multiple myeloma; those who tended to lose the lighter ^{42}Ca isotope, also seemed to be the patients with a more active form of the disease.

"The big advantage of these measurements is that they show what is happening in the bone in real-time, whereas traditional bone health measurements, such as dual-energy x-ray absorptiometry (DXA) scans, show what has happened, when damage may have already been done," claims Ariel Anbar, President's Professor at Arizona State University. The advantage of performing a simple urine or blood test, rather than undergoing x-ray testing, is obvious too.

It's hoped that this new method may also evaluate and optimize the efficiency of bone-specific therapies in the future. Anbar, a geochemist, now wants to bridge the understanding gap between geochemists, and biomedical researchers, who may struggle to understand the test or inaccurately believe it to involve radioactive isotopes. "Closing this gap is vital for developing the technique," says Anbar. *JR*

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In My View

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

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

Contact the editor at fedra.pavlou@texerepublishing.com

The Digital View

Manual microscopic analysis is the gold standard for analyzing blood smears, but it's time to make way for automated microscopy

By Giuseppe Lippi, associate professor, Laboratory of Clinical Chemistry and Hematology, University Hospital of Parma, Parma, Italy.

For decades, we've been counting and measuring blood cells by analyzing peripheral blood smears stained with May-Grünwald Giemsa or other appropriate stains using a microscope. It's a labor-intensive and time-consuming procedure that requires intensive training and you need technical expertise to interpret what can be seen in the blood smear. It is also plagued with inter- and intra-observer inaccuracy. In the modern laboratory, fully automated hematological analyzers (or hemocytometers) take care of the largest part of the workload. Such apparatus enable quick and accurate blood counts together with classifying the normal and pathological blood cells, which overcomes most of the drawbacks of microscopic analysis (1).

Despite these considerable developments in blood analysis equipment, most hematological analyzers are not capable of accurate classification of all the normal and pathological cells that may be present in blood, so we still need to prepare a number of samples for optical analysis and manual interpretation.

But, it looks like our burdens will get lighter! Recent technological advances have made it possible to introduce automated image analysis systems that connect to hematological analyzers and other laboratory equipment. These automated instruments prepare blood films (wedging and staining the samples on glass slides) using customized criteria obtained from the complete blood count. The slides are scanned and digital blood smear images captured at high magnification. Images are then analyzed using artificial neural networks according to a pre-set database of blood elements. Importantly, the database is customizable and local users can update it, so it offers flexibility.

Another exciting feature is the ability for the operator to modify image size, magnify single parts, accept the actual categorization of blood cells or else shift some elements to other categories (2). This enables the categorization of white blood cells in normal elements

"Despite these considerable developments in blood analysis equipment, most hematological analyzers are not capable of accurate classification."

1.	Standardized approach to cell classification
2.	Transmission of digital images to skilled hematologists in various locations
3.	Storage of a large number of digital images
4.	Training tool for students and laboratory professionals
5.	Fully automated selection, preparation, staining and capturing of blood film images
6.	Screening of potentially unsuitable specimens

Table 1. Advantages of automated microscopy in laboratory medicine.

or atypical leukocytes (immature cells, blasts, variant form lymphocytes). The system will also generate additional information about erythrocyte and platelet morphology, flagging samples for the possible presence of anysocytosis, sickle cells, schizocytosis, spherocytosis, acantocytosis, large platelets and platelet aggregates among others (3).

It is unlikely that automated microscopy will completely replace human eyes, but there are many clear benefits, as well as other less obvious advantages, emerging (Table 1). From my clinical perspective, automated image analysis systems allow a standardized approach to cell classification, so that you can compare the digitized blood-smear image with reference slides making the diagnosis consistent with the current morphological classification of hematological malignancies and associated disorders.

These systems also allow less skilled operators to send a digitized blood smear by email or via the web to expert hematologists to get their support and interpretation – they don't need to be in the same location, which is a huge benefit for small or stat laboratories. Another important advantage is they enable digital storage of large numbers of images for each patient, which allows a more accurate longitudinal comparison of data in follow-up and therapeutic monitoring. Such images have a secondary – yet important – benefit because you can project them onto a large screen for training students and laboratory professionals, making it much easier to share knowledge and teach across the group (2,3).

"Although there have been many attempts to identify hemolyzed specimens, it remains a major challenge for laboratory hematology."

There are also important cost savings with automated microscopy systems. For instance, you can optimize the software to identify suggestive abnormalities without the direct intervention of an operator, which obviates the need for optical scrutiny. And, because the whole process of selecting, preparing, staining and capturing the blood film is automated, you reduce the turnaround time and save on human resources.

Another valuable benefit: it can alleviate preanalytical problems, which are the main source of laboratory errors and diagnostic delay in clinical chemistry and hemostasis testing. Among these, hemolyzed specimens are the leading cause of sample rejection and test suppression (4). Although there have been many attempts to identify hemolyzed specimens, it remains a major challenge for laboratory hematology. Recent evidence does suggest that automated image analysis systems may help with detecting a number of abnormalities in the blood film that are frequently associated with red blood cell injury (for example, the appearance of cellular debris, anisocytosis, increased size and heterogeneous shapes of platelets), and this could be used for screening sample quality (5).

So, despite being the gold standard, microscopic analysis of blood smear carries a number of technical and practical drawbacks that can be at least in part overcome with automated microscopy. We just have to embrace it to begin to realize the benefits!

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Genomics for Pathologists

A new approach aims to answer the urgent need for genomic pathology education

By Richard Haspel, assistant professor of pathology at Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA.

It took more than 10 years and billions of dollars to sequence the first human genome. Now, genomic testing has entered clinical practice, and areas as diverse as prenatal testing, microbiology, and oncology use next-generation sequencing methods. Even if they're not directly overseeing molecular pathology laboratories, all pathologists are involved in clinical diagnostics and will be involved in genomic testing. For example, it is increasingly common for tumor specimens to require gene panel testing or even whole-exome analysis to determine treatment options. Even if an outside laboratory does the testing, it's crucial for the pathologist to ensure appropriate sample identification, selection, and processing, as well as that the correct genomic test is ordered. If a sample with mostly normal tissue is sent, no matter how expensive and advanced a sequencer is used, the results will not be helpful ("garbage in, garbage out"). Furthermore, all results need to be integrated into the context of the histology report.

As such, I believe that pathologists must understand genomic testing. In 2010, a survey of residency directors in the USA – conducted through the Program Directors Section (PRODS) of the Association of Pathology Chairs – revealed that only 30 percent of residency programs incorporated any genomic pathology training. In response, through PRODS, the Training Residents in Genomics (TRIG) working group was formed to develop teaching aids and promote the importance of genomics education. From its inception, there was a uniquely collaborative approach within the group. Whereas many curricula are designed by single organizations in a single specialty, the TRIG working group includes experts in molecular pathology, educational design, medical genetics and genetic counseling.

"Even if they're not directly overseeing molecular pathology laboratories, all pathologists are involved in clinical diagnostics and will be involved in genomic testing."

Many education committees also stop at a list of competencies, without providing tools for implementation. So far, the TRIG working group has held 10 workshops to educate both practicing pathologists and pathology residents. These sessions use state-of-the-art teaching methods such as "flipped classroom" and "team-based learning (TBL)." Attendees bring their laptops and work in teams to answer clinical questions using online genomics tools. These highly rated sessions focus on a single breast cancer patient and topics include single gene testing, multi-gene panels and whole exome sequencing (1).

With funding from a National Cancer Institute R25 grant and education design support from the American Society of Clinical Pathology (ASCP), a workshop instructor handbook and toolkit have been developed to help others implement some or all components of the eight-hour curriculum. The 80+ page handbook contains workshop questions and answers as well as detailed information on teaching using the flipped classrom and TBL formats, preparation checklists, and tips for implementation. The toolkit has all the necessary handouts and PowerPoint lectures. Both the handbook and the toolkit are freely available at www.pathologylearning.org/trig. Since their release in December 2014, more than 200 individuals from 15 different countries have downloaded the materials.

Evaluation is a critical component of any curriculum. Since 2014, the TRIG working group has incorporated genomic survey and knowledge questions on the pathology resident inservice exam (RISE) (2). This exam, administered by ASCP, is taken by almost all pathology residents in the USA. An evaluation on such a large scale is unusual in medical education and provides a comprehensive picture of current pathology resident genomics training. The results from the 2014 exam show that program directors are recognizing the importance of genomic pathology, with almost 70 percent of the more than 2,500 residents surveyed reporting some training.

Future goals for the TRIG working group include developing and assessing

"I firmly believe that all pathologists need to understand how to apply genomic methods."

online modules. This will translate the in-person workshop learning experience into a virtual environment. In addition, workshops and courses continue to be held. There will be a three-and-half-hour course at the College of American Pathologists annual meeting (October 4–7, 2015, Nashville, TN, USA). The ASCP annual meeting (October 28–30, 2015, Long Beach, CA, USA) will feature a four-hour workshop for all attendees and an eight-hour workshop specifically for residents. There will be a similar resident workshop at the United States and Canadian Academy of Pathology Annual Meeting (March 12-18, Seattle, WA, USA). A "train-the-trainer" workshop is also being held at the American Society of Human Genetics annual meeting (October 6–10, 2015, Baltimore, MD, USA) to allow people who may teach this material to develop some expertise in flipped classroom and TBL methods.

I firmly believe that all pathologists need to understand how to apply genomic methods to patient care. The TRIG working group is making this possible through its unique and effective approach to genomic pathology education – an approach that can also be applied to other topics and specialties to benefit medical education on a larger scale.

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Companion Diagnostics Champions

At the Poundbury Cancer Center, pathology, business and quality assurance are uniting to create a cutting-edge home for companion diagnostics.

By Roisin McGuigan

The Magnificent Seven. The Avengers. The A-Team. Fiction, and often reality, are rich with examples of teams that use the unique abilities of their members to accomplish much more together than they could have done alone. This year in Dorset, UK, three very different groups in the healthcare arena are teaming up and pooling their talents to tackle a huge challenge facing pathology and healthcare: the development, standardization, and dissemination of companion diagnostics for cancer therapies.

Targeted cancer treatment is currently one of the most exciting and fastest-growing areas in healthcare, and as therapy options expand, so too do the range of corresponding tests needed to guide oncologists down the correct treatment path. But with the deluge of new diagnostics and treatments, it's understandable that pathologists and clinicians alike might have trouble keeping up to date. The constant threat of diminishing finances is also ever present, and these challenges combined can lead to slow uptake of new tests, concerns regarding their proper use and interpretation – and ultimately, failure to deliver the optimal treatment to patients.

Enter the Poundbury Cancer Institute (PCI); a center specializing in companion diagnostics for targeted treatment. PCI opened its doors in May of this year, with high hopes of positively impacting the companion diagnostics landscape through research and education. And within its walls, an ambitious collaboration is taking place between the UK National Health Service (NHS), industry, and quality assurance services.

We speak with key members of this diagnostic development trio: Corrado D'Arrigo, pathologist and co-founder of PCI; Keith Miller, quality assurance expert; and Christopher Hudson, Roche UK Director of Tissue Diagnostics. All three aim to merge their know-how and resources to accelerate the acceptance of companion diagnostics in labs around the UK. But can industry and a national health service work alongside each other in harmony or through gritted teeth…?

Putting Pathology at the Heart

Poundbury Cancer Institute aims to put pathology at the forefront of cancer research, and bring industry, national healthcare service and quality assurance together to expand the use of companion diagnostics and important biological markers.

By Corrado D'Arrigo

The path to implementing a new companion diagnostic test is often a long and difficult one: lack of familiarity with a test, inadequate knowledge and training, and inability to acquire funding, can all foil tests before they even make it to the hospital lab. I co-founded the Poundbury Cancer Institute (PCI) with the sole aim of alleviating many of these issues, and accelerating the adoption of companion diagnostics and other important lab tests.

What led me here?

My interest in cancer biology traces back to medical school, and during my postgraduate histopathology training I started getting involved in research. My career later led me to become senior lecturer at the Hedley Atkins Breast Unit in London, where I was also consultant pathologist at Guy's Hospital, and director of the Breast Tissue Bank. When I left London to work at Dorset County Hospital (DCH) in Dorchester, I used my experience to set up a lab, which focused on innovation to provide a modern, and high quality diagnostic service.

During my time at DCH, I initiated a collaboration with the UK NEQAS (National External Quality Assessment Service) that resulted in a pilot project to translate landmark findings – already well-supported by peer-reviewed research – into clinical practice. The importance of some of these findings, for instance the assessment of microsatellite instability in colorectal cancer (CRC), have been known for at least two decades. Others, such as determining BRAF mutation status in melanoma to predict response to targeted therapies, are more recent findings. We teamed up with the multinational diagnostics company, Roche, and worked on a number of areas – including the establishment of routine prognostic and predictive tests for CRC (see Sidebar "Colorectal Cancer Collaboration: A Case Study"), prostate cancer and melanoma diagnosis, and the routine use of multiplex staining. We succeeded in establishing new molecular pathology services that can be delivered even by the histology departments of small district general hospitals, such as a practical molecular classification for CRC patients that necessitated the creation of an effective workflow through the lab, to meet the demands of a clinical service. Our success meant that we received numerous requests for more projects, but we simply weren't set up to take on board the additional volume of work, and more importantly, we weren't equipped to deliver the necessary training of lab staff and pathologists. I decided, along with my colleagues, to establish a laboratory and teaching center better suited for the challenges ahead – PCI.

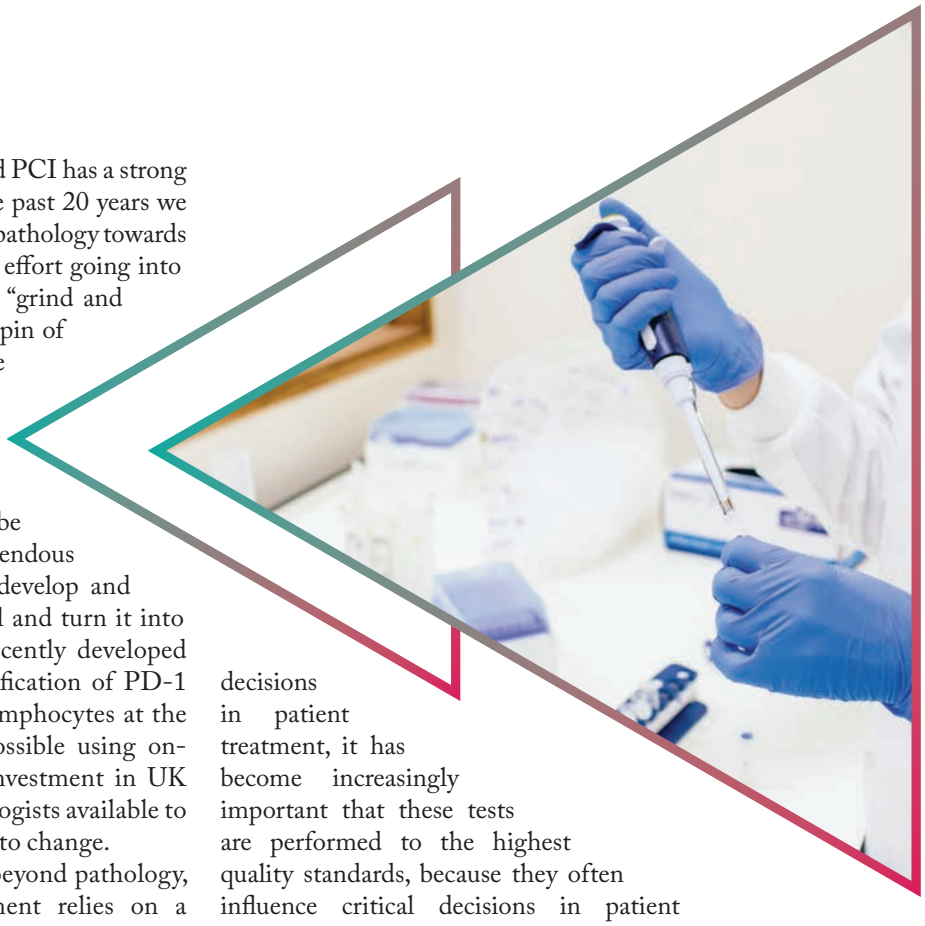
Winning the trust of the NHS

Funding was never a major issue for us; PCI was set up using a mixture of private investments, commercial funding and charitable donations. But creating the Institute didn't come without its problems. Paradoxically, gaining acceptability from the UK National Health Service (NHS) has been difficult. We found that, as an external, private institution, there is an assumption that we want to centralize testing and take work away from public labs. On the contrary, we want to support them and facilitate local introduction of the tests that we develop. We now have a network of NHS labs that want to collaborate with us, and we hope to expand this further. But despite this, I believe our main challenge is to ensure that our scope and functions are understood by the very hospitals and staff our work is intended to benefit.

Building our relationship with the NHS is essential, in particular because PCI has been developed to facilitate teaching and translational research. To do this, we are creating a small clinical service, including molecular diagnostics, to be made available to local patients. A digital microscopy classroom will be built and used to train pathologists, and biomedical and clinical scientists in the interpretation and quantification of cutting-edge diagnostics. Updates for oncologists and surgeons will also be available, to make sure that they are aware of new developments, and can interpret the results that we provide. We intend to inform patients, too – by providing information and holding lectures on advances in the detection and treatment of their diseases.

Reevaluating the pathologist's role

Although the Institute will cater to both clinicians and patients, laboratory medicine will have a central role in our work. My colleagues and co-founders Teresa Thomas and



Saleem Taibjee and I are all pathologists, and PCI has a strong focus on tissue diagnostics. However, in the past 20 years we have seen a progressive shift away from histopathology towards molecular biology and genetics, with much effort going into developing systems based on the so-called "grind and find" approach. Pathologists, once the linchpin of much of the progress in cancer research, have been confined to a diagnostic role.

But things are changing – renewed interest in the localization of molecular changes within the tissue microarchitecture has caused this role to be revalued. Histological slides contain a tremendous amount of information, and we need to develop and refine techniques to interpret this material and turn it into benefits for our patients. For example, recently developed checkpoint inhibitor drugs require quantification of PD-1 and PDL-1 staining in tumor cells and lymphocytes at the tumor-host interface, and this is only possible using on-slide tests. Sadly, I believe that lack of investment in UK histopathology has resulted in fewer pathologists available to support these innovations – and this needs to change.

It's important, however, for us to think beyond pathology, though: successful diagnosis and treatment relies on a multidisciplinary approach. We need the expertise and advice of our surgeons, physicians and oncologists in order to identify diagnostic areas that could benefit most from further development; the formulation of molecular classifications and the identification of appropriate risk groups needs the support of all disciplines.

"Lack of investment in UK histopathology has resulted in fewer pathologists available to support these innovations – and this needs to change."

Assuring quality

Another important collaboration within PCI is our work to support CADQAS (Cancer Diagnostic Quality Assurance Services), an independent, not-for-profit, community interest company. Since most new diagnostic tests influence critical decisions in patient treatment, it has become increasingly important that these tests are performed to the highest quality standards, because they often influence critical decisions in patient treatment. External QA plays a crucial role in this. CADQAS works with key opinion leaders in the UK and beyond to support the introduction of slide-based companion diagnostics and to help all UK NEQAS participating labs make improvements to their current practice. Additionally, CADQAS engages with industry at an early stage, so that these programs can be developed ahead of the launch of the corresponding targeted therapies. Providing support for CADQAS is a key objective that is recognized within PCI.

Industry expertise

This brings us to the other key player in our collaborative model – industry, and in particular, Roche. I believe their involvement brings many benefits to our work. In the past, the academic community had an important role in the development of tests, but industry has increasingly emerged as the major provider, especially in companion diagnostics. Working with Roche allows us to benefit from their considerable experience in performing and interpreting new tests, and in successfully transferring these technologies to clinical practice. Additionally, bioscience companies often have access to technology not yet on the open market, and they can make this available to their collaborators in order to accelerate development.

These benefits don't come without challenges: the NHS is typically wary of industry, and fears opportunism rather than symbiosis. On the other hand, the private sector needs to ensure these partnerships are fruitful for patient care, and these interactions should not be viewed merely as a means for increasing revenue.

"These benefits don't come without challenges: the NHS is typically wary of industry, and fears opportunism rather than symbiosis."

Dispelling suspicions

The NHS might be suspicious of industry intentions, but they stand to benefit from these collaborations. Adoption of new technologies by the NHS can be slow, and the obstacles many. In general, the situation is much better in countries with insurance-based healthcare systems, such as the US or Germany. For example, when the FDA approves a new treatment, the approval and reimbursement associated with the necessary companion diagnostic are arranged simultaneously. In the UK and numerous other European health systems, there is a less structured provision for reimbursement. Some pharmaceutical companies provide free companion diagnostics for an initial period of time, and this encourages early adoption, but these tests are often handled by central labs, and little effort is made to ensure local hospitals can perform them. So once the free period ends, labs are left without the support or funding they need to implement the test themselves.

CADQAS aims to aid NHS labs with these issues. An important remit of the Institute is to support local labs in performing tests, interpreting results and using the data to plan treatment. We will also work to assess the health economics of diagnostics, and provide managers with data that helps them identify funding. Often, we find that new tests not only improve the quality of service but also reduce costs for the hospital – this kind of data is invaluable to managers.

Our relationships with other labs, both in the private and public sector, will allow us to disseminate training and technologies as quickly as possible. In the initial phase of test development, we plan to provide a remote service to our local clinicians, making sure treatments can be used as soon as they become accessible. This has the added benefit of allowing us to optimize workflows, improve efficiency, and troubleshoot tests. We can also examine interpretation, and identify the best format to communicate results to clinicians and patients; for a new test to become established, it is crucial that clinicians understand its context and how it can instruct treatment. Aided with all this practical knowledge, we can then support local labs in introducing these tests to their routine.

Increasing accuracy and improving outcomes

Although support and training will form an important part of the work at PCI, research will be a focus, too. We plan to develop tissue-sparing multiplex staining wherever feasible, to ensure sufficient tissue is preserved for use with companion diagnostics. The pharmaceutical industry is already combining targeted therapies to increase their effectiveness and prevent the emergence of resistant disease. Already, chemotherapy and radiotherapy have replaced surgery as the primary treatment of choice in some forms of low rectal cancer, and it's possible that surgery will increasingly be replaced as the first-line treatment. Potentially, this will mean more detailed molecular assessment of each individual patient is needed ahead of initiating any treatment, and we need to ensure that pathologists are able to deliver this data. With increasingly accurate tests for predicting the biological potential of each cancer, we can offer patients better cancer management, and more personalized treatments – helping us to further the Institute's key operating principle: to help the NHS improve outcomes for cancer patients.

Corrado D'Arrigo is a consultant histopathologist at Dorset County Hospital, UK, and the co-founder of Poundbury Cancer Institute.

Colorectal Cancer Collaboration: A Case Study

Back in 2012, before teaming up for PCI, Corrado D'Arrigo, UK NEQAS and Roche Diagnostics collaborated on a colorectal cancer (CRC) program, the success of which kick-started talks of a more long-term partnership, which has now been realized in Poundbury.

What inspired the first collaboration? The clear need to improve CRC testing. Traditionally, prognostic testing was restricted to staging; not the most precise of models. And while a number of well-supported tests have now been developed for molecular profiling of the condition, these tests are often only available in large institutions, or as part of research programs and clinical trials. In cases where local hospitals do have access, samples usually need to be sent away, which can result in long turnaround times.

The aim of the alliance was to introduce new, efficient and accurate tests to small NHS hospitals. It involved setting up an integrated workflow to ensure all individual tests could be performed together on a sample (as opposed to the common practice of batching several different samples that require the same test), without disrupting the work of an already busy diagnostic service. A classification system was also devised, to communicate the information gained from testing to clinicians.

The work of the partnership team resulted in the creation of a panel of predictive and prognostic tests (see Table 1) that aids oncologists in separating the various disease entities that fall under the umbrella of CRC, helping them to choose the most appropriate treatment. Some of the tests are now seeing more widespread use – for example, the 2014 RCPath revision of the minimum dataset for reporting CRC now includes microsatellite instability (1).

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Colorectal Cancer Panel – What Tests and Why?

MSI	Mutation Analysis for EGFR, KRAS, BRAF, NRAS and PI3K	Loss of PTEN	HER-2 Amplification	Loss of CDX-2, Loss of CK20 and Expressionof CK7
• Numerous studies have shown that MSI tumors have a better prognosis, do not benefit from 5-FU based adjuvant chemotherapy, and respond well to combination therapy such as anti-VEGF and oxaliplatin (2, 3). • Recent data has also suggested that MSI patients may benefit from the checkpoint inhibitor anti-PDL-1, while MSS patients do not respond to this class of drug (4).	• There are a number of new treatments such as cetuximab, panitumumab, regarofenib, and more which rely on these tests to predict responses (5). • BRAF in combination with MSI also separates familial from sporadic disease (6).	• There is evidence that loss of PTEN may predict a lack of benefit from anti-EGFR therapy in metastatic CRC (7); its prognostic role in CRC is currently debated. • Some observed discordances may result from inconsistent analysis and interpretation. • PTEN loss has been added to the panel to work towards standardization.	• The Heracles trial has resulted in renewed interest in HER-2 amplification. • The study trialed lapatinib and trastuzumab in patients with HER-2 amplified, KRAS wild type metastatic CRC, with tumors that no longer responded to chemotherapy and anti-EGFR therapy, with positive initial results (8).	• Prognostic factors associated with aggressive clinical behavior. • CRC patients with simultaneous loss of CDX2 and CK20 expression in tumor tissue constitute a highly aggressive subgroup of MSI CRC patients (9).

Table 1. The CRC panel and its rationale. Developed by a collaboration between staff at Dorset County Hospital, Roche Diagnostics and UK NEQAS. MSI, microsatellite instable; MSS, microsatellite stable; 5-FU, 5-Fluorouracil; PDL-1, Programmed death-ligand 1.

Industry Insights

Christopher Hudson is Director of Tissue Diagnostics UK and Ireland at Roche, the multinational life sciences company that supports the Poundbury Cancer Institute through funding and research collaboration.

What are the challenges when introducing a new companion diagnostic to clinical laboratories?

Development is a long and complex process, and in the UK, adoption can be very slow – it can take years before a test for an approved therapy sees widespread clinical use. There are many reasons for this, but in my opinion, the biggest obstacles fall into three categories:

1. Time – Many sites want to perform their own studies on a new diagnostic, which, given the regulatory hurdles the therapy and test have already gone through, can unnecessarily duplicate efforts. Establishing external quality assurance, and ensuring training, validation and optimization of a test can take some time. Finally, allocation and wrangling over funding can delay uptake, too.
2. Funding – In vitro diagnostics are not reimbursed in the UK, but instead funded through block contracts, or through national or local treatment tariff arrangements. Pathology is often seen as a cost, rather than being seen as adding value to medical decisions. But while only 2–3 percent of the total healthcare spend is made in this area, the vast majority of therapy decisions are made on the basis of test results. In the absence of a sustainable national framework for delivering companion diagnostics, pathology departments have to manage introduction of these relatively expensive tests within their existing budgets; this is a huge barrier to getting new treatments into the clinic. At the same time, failing to introduce these tests can result in costly and potentially unnecessary treatments for patients (Figure 1).
3. Resources – Over the years, the role of pathologists seems to have become subservient to that of clinicians, and the balance of available resources between the two groups seems completely out of kilter. Pathology needs to be appropriately resourced, with a workforce skilled in both testing and interpretation, in order to be able to give clear information and direction on the application of therapies.

How can these issues be overcome?

In the US, we're noticing that the FDA is starting to accelerate the approval of therapies with companion diagnostics, because of the clear benefits to patients of getting targeted therapies to market. Here in the UK, it is imperative that we put the appropriate framework in place to facilitate a similar rapid adoption.

Precision medicine heralds a new dawn in healthcare – and companion diagnostics are as critical to the application of precision medicine as the drugs themselves. This evolving area provides an opportunity to redefine the landscape, role and value of pathology, in particular histology. The time has come for pathologists to take the initiative, and to start leading these discussions – it would be great to see the Royal College of Pathologists proactively addressing these issues with their colleagues. Pathologists, clinicians, patient representatives, quality assurance schemes and finance departments all need to work together to provide a more balanced approach to the allocation of resources.

At Roche, I see our role as being both a strategic and operational partner to pathologists and laboratories. We already work closely with histology labs, supporting them with activities such as training, education, assay optimization and technical support. And providing the appropriate training is critical – medical practices are changing, and pathologists have to be prepared to accurately interpret increasingly complex results and advise clinicians accordingly. CADQAS and Poundbury have a clear mission to provide the training the pathology profession needs, and this is one of the reasons why we have chosen to work with them.

What benefits will Roche bring to PCI?

Roche has made a several year commitment, in the form of ongoing funding for the Institute's work. We are providing instruments and reagents free of charge to PCI to help them undertake their work within the UK lab community. In addition, PCI has strong connections to our teams in Europe and the USA, where development of diagnostics takes place.

With the wave of new companion diagnostics getting ready to hit the UK, pathologists and labs need support. The chronic lack of pathologists, coupled with their ever-growing case workloads, means many of them simply don't have the time to research multiple potential new approaches and their applications. PCI will help pathologists by educating and

training them in the use of these techniques. Investigating where and how new tests will fit into treatment pathways is crucial, and by working with us early in the development cycle, PCI will have access to new companion diagnostics while they are still in development, and they in turn can prepare pathologists well in advance of the tests being released.

"This evolving area provides an opportunity to redefine the landscape, role and value of pathology."

What does Roche gain?

This is the first such collaboration Roche has made in this area – the company will be watching closely to see how the relationship develops, as this could potentially become a blueprint for further similar projects. Our work with PCI and CADQAS will provide invaluable feedback on the process of bringing new companion diagnostics to the UK, including issues such as EQA schemes, training, and support needs. So far, getting new biomarkers and their corresponding tests into routine clinical practice has been slow. With the help of PCI, it should be possible to speed up adoption, benefiting our stakeholders and the NHS.

Is the NHS cautious of industry involvement?

I am always saddened when I hear members of the NHS refer to industry in less than glowing terms, but I can understand their point of view – not all companies are created equal, and a previous negative experience may be off-putting. But they shouldn't let the actions of a few tarnish us all. I really don't believe in an "us and them" culture – it's not productive in the long-run. Industry needs the NHS, and the NHS needs industry. Although we may have some different viewpoints and perspectives, there are areas of common ground where we can easily work together to benefit patients. We won't always agree, but with an honest, open dialog, I believe we can go far.

We are delighted to have been invited to work with PCI. This is the culmination of years of discussion, and we have high hopes for the future – PCI is a shining example of what can be achieved when the NHS and industry work together for the greater good.

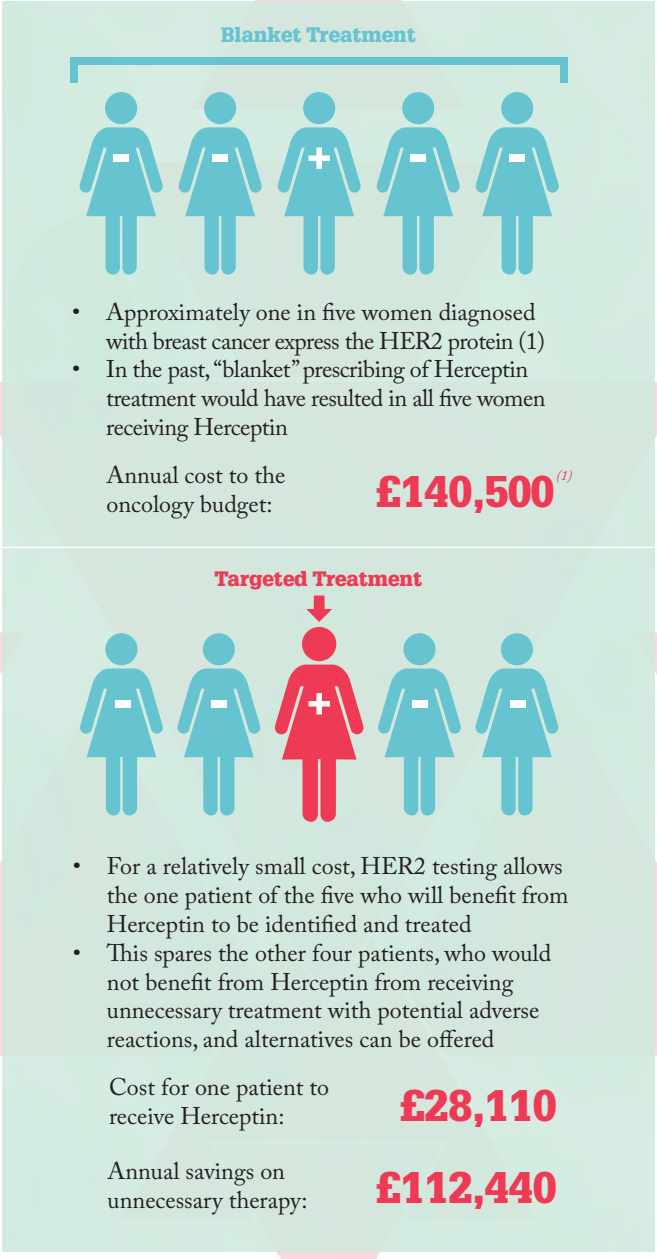

Figure 1. The High Costs of Untargeted Treatment
Breast cancer survival rates are improving, with five-year survival increasing from 80 to 87 percent within the last decade, largely due to improved therapies. HER2 is an example of the progress being made, and the benefits of precision medicine.

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Quantifying Quality

Keith Miller is Director and co-founder of CADQAS (Cancer Diagnostic Quality Assurance Services), a not-for-profit community interest company hosted by Poundbury Cancer Institute (PCI), which works to provide information and training to laboratory staff, and to ensure the quality of cancer diagnostics.

What led you and Sarah Wedden to found CADQAS?

My career has been a great grounding for this project – I was very fortunate to be at the heart of the developmental work on immunohistochemistry (IHC) and in-situ hybridization (ISH) back in the 1980s, under the guidance of one of the world's leading academic researchers in the field at that time, Professor Peter Isaacson. Applying these technologies to cancers previously diagnosed using morphology and tinctorial stains revealed that many of the old diagnostic methods were seriously flawed. Developments like IHC allowed cancers to be more reliably classified, which meant a growing number of tailored treatments could be applied – IHC and ISH testing have underpinned much of the improvement in cancer care today. And in some patients, the response to tailored drugs (for example, giving Herceptin to HER-2 positive breast cancer patients) can be phenomenal.

Since 1985, I have also been involved with external quality control for IHC and ISH, after being invited by those who initially started the activity – Gerry Reynolds (Mount Vernon Hospital), and Brain Mepham (Southampton General Hospital). In 1988, this work was reviewed and was recognized by the Department of Health, and subsequently included as part of the UK National External Quality Assessment Service (UK NEQAS). The scheme oversees the quality of IHC and ISH testing performed in diagnostic laboratories in both the UK and 50 other countries, and supports labs struggling to meet the appropriate standards. As scheme director, part of my remit is to ensure that there are appropriate training programs available for laboratory staff to update their skills. The scheme also has a duty to give staff who are having testing issues somewhere to go in order to discuss their problems, and work to address them. In my opinion, the changing healthcare environment in the UK has caused laboratories to become more and more competitive. This means the "help thy neighbor" approach is quietly disappearing, while at the same time, the cost of running courses in many locations has become prohibitive. These issues make training and QA programs more important than ever.

So, when the opportunity, contributed to in part by a generous donation from Roche, arose to set up an independent, "not for profit" community interest company, to support the education and training of laboratory staff, Sarah Wedden (co-founder of CADQAS) and I grabbed it! Poundbury provides an exceptional environment for our work – and my background in tissue testing and QA gives me a wealth of experience to draw on in this new role.

"The changing healthcare environment in the UK has caused laboratories to become more and more competitive."

What role will CADQAS have within the Institute?

Before CADQAS, there was already work going on to support the NEQAS program in the nearby Dorset County Hospital. This was at the invitation of the lead pathologist, Corrado D'Arrigo. It was through funding from Roche Diagnostics that we were able to appoint a very able research scientist, Sarah Wedden. Sarah and Dr D'Arrigo have worked closely together since early 2013 on projects in colorectal cancer (CRC), prostate cancer and melanoma. The information they have produced is very informative, and they have already given presentations both in the UK and overseas. Although not cutting edge science, this educational and supportive work is crucial for labs that are struggling to keep up with the demands of the 21st century.

Improving the approach to cancer testing is also the cornerstone of how CADQAS will work within PCI – a number of vitally important markers, for instance in CRC, are not universally embraced, which can lead to patients failing to receive the right treatment. Adoption of the new and novel diagnostics can often be slow, and there are already examples of potentially life-saving tests which are not being used by a significant number of cancer testing centers, such as EML-4Alk and ROS-1 testing for lung cancer.

CADQAS will help both by offering training and support, and in the future, we aim to help improve the diagnostics themselves. Both our company, and our network have considerable experience in this field. Combined, we can influence change for the better. From a QA angle, we will also be involved in investigating issues with tests – for example, should a batch of reagents fail, we will work to find out why.

Clearly, Dr D'Arrigo, Sarah and I do not have all the necessary know-how to deliver truly comprehensive teaching programs. We hope to bring experts from the UK and beyond to PCI to lend their expertise to our courses. We want to involve industry, too, and are already speaking to a number of pharmaceutical and analytical companies about our work. We aim to create programs that benefit everyone in the field, but above all, lab staff and the patients they serve.

What challenges do you anticipate, and how will you overcome them?

The UK NEQAS Scheme for IHC and ISH has already successfully improved HER-2 testing for breast cancer in the UK, and breast cancer hormonal receptor testing has greatly improved, too. As research and clinical practice evolve, we need to continue to safeguard the quality of the assays being performed, as well as the interpretation of results.

For example, we are currently discussing ways to support the introduction of PDL-1 testing into the community, and how to train and provide robust external quality control systems. PDL-1/PD1 therapy is likely to significantly improve outcomes in a range of cancers, including lung, skin, bladder and breast. The companion diagnostics for these treatments are likely to be provided by just two suppliers, for the four different check-point inhibitor targeted therapies. Potentially, this means four assays, with four different interpretations. This represents an immense challenge – each assay must be interpreted as accurately as humanly possible, and at the same time we are reliant on the assay providers to provide consistency from batch to batch.

However, our laboratory is now starting to have access to some of these assays, and we are in a position to provide a range of cancer cases that will be stained with the relevant IHC companion diagnostic on glass microscope slides. We can then digitize the slides in high resolution, and create a portable, digital teaching set, and give lab staff, both medical and scientific, the knowledge they need to interpret tests correctly.

I am proud to say that the quality of IHC and ISH performed in UK laboratories is among the highest in the world today. But with new and highly complex diagnostic markers on the way, our external QA system will be severely challenged if we don't take the necessary steps to support the related companion diagnostic in the field – something that is absolutely imperative if we are to make sure that every patient gets the right therapy for their cancer.

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The Future Is Here! But How Do We Deal With It?

Molecular diagnostics provide us with huge amounts of data – but, as a profession, we're still working on the best ways of collecting and using it

By Mathew Diggle

Change is everywhere in pathology. With each newsletter, magazine or journal article, we're seeing new technology and new applications for it – faster sequencing, more efficient sample preparation, cheaper software, and better ways of acquiring a lot of information at minimal cost. But while all of these advances are valuable, I think that value may be reaching a plateau. What does that mean? To me, it means that we're generating information that is so detailed and so extensive that we're unable to take full advantage of it. In essence, it means that we now have access to more information than we can use – so if more data isn't the answer, how do we keep advancing? We need to look beyond technological improvements to determine how laboratories can make the best and most efficient use of the wealth of information now available to them.

At a Glance

- *Molecular diagnostics give pathologists more information on patients and diseases than ever, but more isn't always better*
- *The increased volume of data can lead to confusion, prompt unnecessary testing, or discourage pathologists from updating their knowledge*
- *Each unique laboratory needs to balance the pros and cons of technological advancement and prioritize the most efficient advancements*
- *To best understand and make use of new technology, we should establish strong lines of communication with other labs and professional sectors*

We've seen huge developments in molecular diagnostics in the past decade. Many technologies that were originally exclusively used within research have now become more refined and user-friendly. At the same time, there's been a marked decrease in the cost of those technologies – a combination that has made them more applicable to the needs of a routine clinical laboratory. When these changes were new, clinical virology laboratories were natural early adopters because the advantages (in terms of time, cost and labor savings) offered by molecular techniques were so overwhelming when compared to traditional techniques like cell culture. But since then, the other areas of clinical microbiology have also begun using genetic and molecular testing to inform their conclusions. And the technology is useful – it shows increased sensitivity and specificity over traditional phenotypic diagnostic methods, and often yields better reproducibility. In fact, "black box" technologies that automate laboratory tasks have shown advantages in reduced hands-on time, quick turnaround time to result and reliability – advantages that have real benefits for patient care and the quality of the support we can provide. The data also help us to better understand infection and prevention in hospital and community settings, as well as giving us the tools to continually improve and challenge ourselves. But if we opt to employ new technologies, especially ones that automate laboratory tasks that were previously our responsibility, we need to ensure that we aren't sacrificing a complete and detailed understanding of the diagnostic process.

"In adopting new technologies, we risk reducing our understanding of the reasons we conduct a given test."

More information, even with regard to identifying and characterizing disease, doesn't always mean a better outcome for us or the patient. It forces us to ask ourselves, "What does this information mean?" And sometimes, having so much data to interpret can even be a disadvantage. Where we might previously have obtained a negative test result, we might now get a positive; where we might previously have had a single positive result, we now have multiple results to interpret and weigh against one another. In adopting new technologies, we risk reducing our understanding of the reasons we conduct a given test. The initial stages of clinical diagnosis, especially the selection of appropriate tests, have been an important part of our role. But if people don't fully understand how and why certain testing is done, it becomes much harder to identify what's truly needed, and we run the risk of "screen" testing, which – if inappropriate – can be as dangerous as not testing at all. Risks like these might make laboratory professionals want to go back to older methods where results are more familiar and interpretation simpler.

A balancing act

Rather than sacrifice the technological gains we've made since molecular diagnostics came to the fore, it's important to work out a balance – and there's no one right way of doing this. Each lab, and indeed each person working in the lab, has different needs and limitations. So if we can't identify a way forward that works for everyone, what can we do instead? Some areas under consideration do apply to every lab – questions of cost, speed, quality, accuracy and precision, and the use of a multidisciplinary approach to take the best possible advantage of the technologies each lab chooses to pursue.

At first glance, speed seems an obvious positive – who wouldn't want a faster turnaround time on lab tests? But the fastest route to completion isn't always the most efficient; not every result needs to be available immediately. There are certainly situations in which rapid testing is valuable, but in many cases, doctors don't review lab test results in a timely manner – or, in some cases, at all (1)! In other cases, even if a test is rapidly completed, doctors may not be able to act on the result with the same speed; for instance, community and general practice centers that request 24- or 48-hour turnaround times for initial results of urine tests may not have the capacity to address the findings in a similar timeframe. So if a provider needs a result in a week's time, why expend resources and delay potentially more critical tests in order to turn it around in half an hour? Though there's no question that speed is a valuable advancement, it's up to us to distinguish the situations where it's truly worth applying from those in which it might not be necessary.

Automation is another gain that seems unambiguous on the surface. But look a little deeper and it isn't always the right approach. The advantages are clear: machines can handle more samples at a time than humans, performing more complex analyses on all of them simultaneously. Handing testing over to an automated platform can save time, preserve resources, and ease the hiring burden on labs already struggling to find staff with the skills they need. And machines can run 24 hours a day, every day, if necessary – something that would seriously cut into pathologists' personal lives! But even with all of these benefits, automation isn't a panacea for laboratory challenges. The recent push toward automated bacteriology, for instance, may not always provide significant benefits compared to manual work – so upgrading might not be worth the time and resources it would take. And not every laboratory has sufficient testing volume to justify purchasing automation technology, especially in the case of newer – and therefore still expensive – devices and software. As

with speed, it's our job to determine when and how automation can be useful, and then to make our case to the people who control the purse strings. Solutions for any given lab might take the form of purchasing new machines, or might involve improving our use of technologies we already own, teaming up with other laboratories to share devices, or outsourcing certain tests.

"Sharing best practices and common challenges has helped us to devise solutions together."

It's true that the costs of molecular diagnostics are dropping rapidly. For some time, they followed a Moore's-law-esque trend of steady decrease; however, after next-generation sequencing eclipsed Sanger-based methods in 2008, the drop was precipitous (2). But as I said earlier, our priorities shouldn't be focused on getting as much data as possible – because with that as our goal, we may end up getting so much information that we can't translate it all into clinical services. It's important to ask ourselves what clinical value any given piece of information might have – and then to make the case for acquiring that information to the people responsible for funding its acquisition. This is especially true when these costs go beyond a single test; for instance, buying a new piece of equipment or hiring a new staff member with specialized skills. Unfortunately, as pathologists, we're often tasked with striking a balance between cost and our other priorities, so it's important for us to be able to identify our needs, figure out how best to meet them, and explain our conclusions to the people in charge of funding.

Step out of the silo

It should be known that these challenges aren't unique to any one lab – you're not alone! It's difficult to establish strategies for advancement when there's no good one-size-fits-all solution to present, but there's a lot to be gained by communicating with other pathologists and learning what they're doing. For labs that are trying to increase or improve their use of molecular diagnostics, I recommend dropping the "silo mentality" we tend to prefer and instead developing a strong network. Share best practices, collaborate with other pathology centers, and break down the divisions between clinical and academic environments. Over the past four years, I've been working closely with colleagues both at my home Nottingham University Hospitals trust and at the University Hospitals of Leicester to implement a consolidated laboratory model (in which local labs reconfigure collaboratively to create greater efficiency) – and it's had significant benefits. Consolidation is difficult, complicated and time-consuming, but sharing best practices and common challenges has helped us to devise solutions together. The same applies to liaisons between healthcare, academia and industry – three sectors that have historically treated one another with some trepidation. There's a lot we can learn from one another, and a lot of opportunities to be gained from making connections between different professional groups.

As a profession, we're getting better at discussion and collaboration, but the culture of isolation and competition is a long-established one and it's difficult to move to a more open way of working. We face the same problem with finances; those pathways aren't currently very transparent, and making them more so would allow us to understand the cost of the complete patient pathway and work toward more efficient improvement by investing in areas that will ultimately result in greater savings or patient benefit. The key is to keep the patient at the center of everything we do. As we start to share more of how we operate – the good, the bad and the ugly – seeing the improvements to patient care should encourage others to participate as well.

The rise of molecular diagnostics, and our need to manage and streamline large amounts of information, has presented us with unique challenges we're still learning to address. But this isn't the first time pathology has tackled such a radical change. In the beginnings of the profession, even identifying the organism associated with a clinical presentation was difficult and confusing – but we didn't step back; we carried on trying to understand, and I see the same thing happening today. As long as we remember that more doesn't always mean better, and focus on using collaboration and prioritization to make things better for our patients, then I think molecular diagnostics will prove to be not only an impressive, but also a useful, tool.

Mathew Diggle is clinical lead for molecular diagnostics in East Midlands Pathology and consultant clinical scientist in clinical microbiology at Nottingham University Hospitals, UK.

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Bridging the Gap in Glucose Monitoring

Glycated serum protein testing has capabilities other blood glucose tests don't, and may expand clinicians' diagnostic and treatment management options

By Timothy Warlow Jr.

Since blood glucose monitoring began in the 1960s, it's been a key parameter for the control of acute diabetes. It has made daily glucose monitoring by patients possible, and, for longer-term control, glycated hemoglobin (HbA1c) assays have traditionally been the primary test used in clinical practice, offering average measurements over a two- to three-month period. But there is a gap which is, as yet, largely unplugged – the gap between short- and long-term testing. Given the exceptionally high prevalence of diabetes, and a projection that the disease will be the seventh leading cause of death worldwide by 2030 (1), it's clear that meticulous blood glucose tracking and management – short-, medium-, and long-term – is of great, and growing, importance. Until recently, there was no reliable marker for medium-term monitoring. But now, a new marker, glycated serum protein (GSP), could be the answer. It could also provide a suitable alternative to HbA1c in patients with abnormal red blood cell turnover.

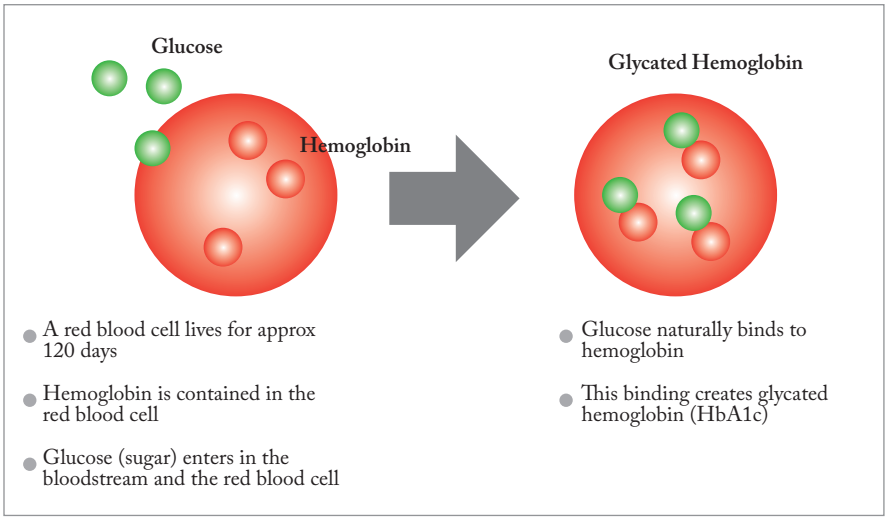

Figure 1. Glucose enters red blood cells and binds to hemoglobin molecules, creating glycated hemoglobin (HbA1c).

At a Glance

- *Glycated hemoglobin (HbA1c) testing, the standard method of long-term glucose monitoring, can be inaccurate in patients with abnormal red blood cell turnover*
- *Glycated serum protein (GSP) levels are similarly reflective of average blood glucose levels and could provide a suitable additional measure*
- *GSP testing is an intermediate marker of glycemia, providing measurements for a two- to three-week period that bridges the information gap between short- and long-term monitoring*
- *Combining HbA1c and GSP testing offers improved diagnostic accuracy and reliability in the prediction of diabetic complications, especially in patients with conditions that affect red blood cell lifespan*

The standard measure for glucose control

Along with daily blood glucose measurements, HbA1c is commonly tested in patients with diabetes because it provides a reliable measure of glycemic control. Circulating blood glucose irreversibly attaches to hemoglobin A in red blood cells, making the hemoglobin molecules highly stable (Figure 1). As a result, HbA1c levels reflect a weighted average of circulating glucose levels over the two- to three-month lifespan of red blood cells, giving clinicians an important piece of information about their diabetes patients' long-term blood glucose management. The HbA1c assay can also be used as a diagnostic test for diabetes, allowing doctors to identify at-risk individuals early and helping them make small lifestyle changes to reduce their chances of developing type 2 diabetes. All of these uses make HbA1c the standard in the diabetes world – for prevention, diagnosis and management of the disease.

> *"Until recently, there was no reliable marker for medium-term monitoring."*

At the moment, HbA1c is primarily used to track long-term trends in blood glucose for diabetic patients; the amount of glycation present on the hemoglobin proteins is measured against the total amount of hemoglobin in the red blood cells. But a technique that relies on

the lifespan of a red blood cell has its limitations. Critically, HbA1c testing is not suitable for patients with conditions that affect red blood cell turnover – hemoglobinopathies, thalassemias, chronic and end-stage kidney disease, and some forms of anemia (2). Factors such as age, race, pregnancy, and certain drug treatments can also skew the HbA1c measurement. In patients like these, an alternative monitoring method is needed.

Closing the gap

Like hemoglobin, serum proteins also undergo non-enzymatic and irreversible glycation. Albumin, also known as fructosamine, is the most abundant of the serum proteins, and glycated albumin (GA) accounts for 80 to 90 percent (3) of the GSP test readout. GSP is also strongly correlated with HbA1c and mean blood glucose in type 1 and type 2 diabetes (4,5). Albumin contains multiple lysine residues that are susceptible to glycation, and reacts 10 times more rapidly with glucose than hemoglobin does (3,6). In addition, it's not influenced by conditions or treatments that affect red blood cell turnover. In fact, GA and GSP have been shown to accurately reflect glycemic control in situations where HbA1c tests are unreliable (6,7).

It's not just an alternative to HbA1c testing, though. At 14 days, albumin's half-life is much shorter than that of hemoglobin, so it provides a unique opportunity to monitor a patient's short- to medium-term glycemic status (6). That means GSP could be used to fill the gap between daily blood glucose testing and long-term HbA1c testing, which is especially helpful in monitoring diabetic patients whose treatment has recently changed or in patients, such as those with gestational diabetes, who need closer monitoring.

When discussing traditional glycemic monitoring, the difference between actual measured HbA1c and predicted HbA1c from GSP is often referred to as the "glycation gap." This gap is a significant predictor of the progression of diabetes complications, including nephropathy and retinopathy (8), and is therefore becoming a useful tool in clinical pathology. It emerged in response to the observation that there are considerable inter-individual differences in HbA1c that aren't explained by corresponding differences in glycemia. Instead, the discrepancy seems to reflect variation in the glycability of hemoglobin across the population (9). Combining HbA1c and GSP measurements to determine the glycation gap should therefore offer better diagnostic accuracy and patient management.

The trouble with tetrazolium

Enzymatic assays for GSP monitoring are more reliable and specific than the traditional method, which uses the chemical compound nitro blue tetrazolium (NBT). NBT can react with endogenous substances that possess reducing activity – like thiol groups, ascorbate and NADH – meaning that it isn't specific to GSP. In fact, studies have shown that only about half of the reducing activity was due to specific glycation of proteins, with the remaining activity varying between samples. Understandably, this lack of specificity limits the accuracy of NBT-based GA assays (10).

Unlike chemical tests, enzymatic methods eliminate the inaccuracies introduced by reducing substances, providing a more accurate and reliable measure of GSP (11). The GSP assay involves three steps, in which two enzymes break down the samples to specifically measure levels of GSP: 1) proteinase digests the glycated proteins into low molecular weight fragments; 2) fructosaminase catalyzes the oxidative reaction of the Amadori products (intermediates in the reaction, which results in protein fragments, amino acids, an intermediate known as glucosone, and hydrogen peroxide; 3) the hydrogen peroxide release is then coupled to a colorimetric Trinder end-point reaction, which is read as an absorbance reading at 546–600 nm. The absorbance value is proportional to the amount of GSP in the sample, providing a GSP measurement for clinical use (11).

"Based on some of the limitations of HbA1c, I believe that GSP testing may be a better marker for glycemic control than HbA1c in some instances."

Based on some of the limitations of HbA1c, I believe that GSP testing may be a better marker for glycemic control than HbA1c in some instances, especially for evaluating glycemic excursions – a major cause of complications in diabetes – and as an alternative to standard testing in patients where the HbA1c test is unsuitable. It also provides an intermediate measure of glycemia over two to three weeks, either bridging the gap between daily and long-term monitoring or used in combination with HbA1c testing to determine the glycation gap. Despite its advantages and the fact that GSP monitoring is available in many countries, it is only routinely clinically used in Japan. I do, however, believe the advantages for clinical pathology are numerous; GSP testing can provide key prognostic information for the prediction and risk stratification of diabetes and

its complications, hence improving the diagnosis, monitoring and treatment of patients with diabetes, the prevalence of which is rising at an alarming rate.

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Welcome to the Molecular Diagnostic Revolution

There's a whole new world of molecular diagnostics out there. Why has innovation been restricted? What are the upcoming challenges for manufacturers? Is it time to take a tip from Mother Nature? Biocartis' Rudi Pauwels has some of the answers.

How far has the field of molecular diagnostics come?

PCR technology essentially established the molecular diagnostic field as we define it today. PCR has been – and still is – a phenomenal research technique, but if you consider how the technology evolved in the laboratory medicine space, much of the innovation focused on automation of the PCR component. Unfortunately, the risk of contamination, among other issues, means that every lab needs dedicated PCR infrastructure, trained technicians, specialized equipment, and so on. The result? PCR technology has not penetrated the space as much as other clinical diagnostic systems, such as biochemistry, hematology, and protein detection.

But every challenge is also an opportunity, which is why a number of companies realized that the next step in pushing this great technology into clinical practice was to take a fresh, holistic approach and taking the entire sample-to-result workflow into consideration. Another key hurdle that needed to be addressed was the need to measure a growing number of biomarkers in a single clinical sample and develop so-called 'multiplexed assay' solutions.

Multiplexed analyses are the way forward?

Mother Nature shows us that it's feasible to simultaneously carry out multiple reactions in a single reaction environment – as long as the system and its components are well designed. Although these multiplexed technologies still have some way to go, the field has in fact made a lot of progress both in terms of system designs and molecular techniques. Advances in next generation sequencing, microarray analysis and multiplex PCR have all contributed to achieving performances incomparable to those of a decade ago. And we're getting better all the time.

But techniques such as multiplexed PCR are just the analytical components of the total solution. As diagnostic technology developers, we must always keep focusing on true diagnostic needs of the physician and patient. The prime diagnostic objective is to provide accurate, reproducible results in close space and time proximity to where patients and physicians interact and first-time-right therapeutic decisions need to be made. This requires new, flexible systems that can process even complex clinical samples directly with minimal manual interventions and without requiring any specific laboratory infrastructure.

Do you feel that innovation has been restricted?

Yes. Molecular diagnosis has traditionally been a technique requiring specialized infrastructure and operators and, as a result, the service has been thus far mostly centralized in high-volume reference labs. In line with that trend, manufacturers built automated solutions for higher volume testing. The flexibility to step away from the traditional batch-based workflows is only a recent development. The challenge of fully automating sample preparation and creating an uninterrupted workflow to analysis has long been underestimated.

Sample preparation was traditionally done as a separate, mostly manual operation necessarily carried out in a laboratory environment. It is well known that manual procedures can be the source of variation and errors in the final results, even with excellent analytical equipment.

How has the lack of innovation stunted growth in personalized medicine?

In most oncology practices, molecular diagnostic results from tumor tissues may take two to three weeks to be returned. These delays, also requiring access to specialized laboratories, not only prolong the anxiety period for patients, but also are an impediment for wider and global adoption of personalized medicine. The true objective of the diagnostic industry should be to develop solutions that can be scaled up on a global level. We need compact, high-performing, high-precision, technical solutions that do not require specialized infrastructure or trained people. Is that enough? Probably not. I think technology is a key enabler of this revolution but there are

other factors. For instance, I am often surprised to hear the debate about the price of diagnostics. Though the high value is – and should be – reflected in the prices of personalized drugs, payers should take a holistic view and also recognize the intrinsic value of the diagnostic solution when considering pricing. *In vitro* diagnostics (IVD) manufacturers and labs should be sufficiently incentivized and rewarded.

It's important to recognize that laboratories, especially the CLIA (clinical laboratory improvement amendments) reference laboratories, play an important role because they can rapidly develop new biomarkers and make them available. I think we should applaud collaborations between labs that can rapidly develop biomarkers and IVD companies that develop the diagnostic solutions, and recognize the value they bring to personalized medicine.

How can new molecular technologies be made attractive to small volume labs?

It all comes down to the current and future needs of the patient, which are complex and often don't involve a single set of symptoms. In this light, the importance of multiplexing capabilities in the platform become more obvious.

Clearly, we also need to develop solutions that are flexible for use in both big laboratories and smaller, point-of-care settings.

How challenging is this approach?

It's a big undertaking. For example, when working on biomarkers that will help stratify your patients for a specific drug, the development of an IVD is expensive and time-consuming. The ideal solution – and the challenge that we have as manufacturers – is to develop fully flexible platforms that use the same basic technology and are amenable to both point-of-care and bigger lab settings. It would lead to a single IVD development of a new assay that can be run in multiple assay volume settings.

Finally, the technology must also be efficient, cater to varying throughputs and skillsets, and be easy to use. No easy task.

Communication and connectivity are also extremely important. When using molecular diagnostic techniques, a lot of very valuable information is collected but is not always fully utilized. As with many other aspects of our life, when things become interconnected it is useful to capture as much information as possible – and in the best way.

I've been very much inspired by Apple and other consumer technologies in terms of developing a flexible molecular diagnostic platform that is designed for a potentially wide range of applications. I therefore want to challenge the older dogma that diagnostic systems need to be designed for specific purposes. If we want high-precision medicine, we will need high-precision diagnostics yielding clinical actionable results irrespective of where or how or by whom the test is performed. From a patient and physician perspective, these technologies are essential.

Can the dream be turned into reality?

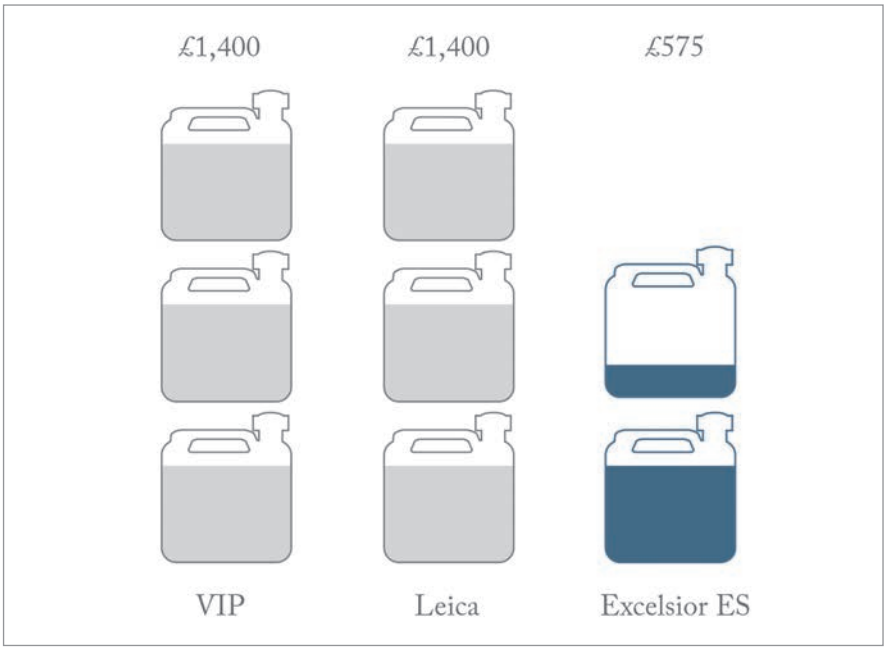
It's our *raison d'être* at Biocartis. And it's absolutely why I created the company in 2007. We have invested heavily in developing a new molecular diagnostic system that goes from sample to results.

However, I think the role of diagnostics is still underestimated. After my years of research in HIV diagnostics and next-generation therapeutics, I moved into industry because I really wanted to make an impact. As an industry, we have high ambition – and I believe we are well on our way.

Rudi Pauwels founded Biocartis in 2007, where he is CEO. He (co)founded several biotech companies, including Tibotec, Virco and Galapagos Genomics. For more than two decades, he focused on the search and development of anti-HIV drugs – a number of which have been approved and introduced on the market – and the development of diagnostic tools to allow personalized HIV treatment.

Reduced Reagent Usage & Reliable Tissue Processing with Thermo Scientific Excelsior

As part of the South Devon NHS Foundation Trust, Torbay hospital serves a population of around 370,000 people. With such a high demand, and the associated targets for turnaround time, it is vital that tissue processing instrumentation is reliable and cost-effective.



A typical comparison of monthly reagent cost of Thermo Scientific Excelsior ES tissue processors versus competitors. Source: Comparative Study, Forum Health – Trumbull Memorial Hospital, Youngstown, Ohio, USA.

In 2004 Torbay hospital made the decision to replace their old, inefficient tissue processors. They trialled all available instruments in the market, with key criteria including reliability, ease of use and reagent change. During the trial the Thermo Scientific Excelsior proved its worth and two instruments were purchased. "It was easier to use and cheaper compared to anybody else's on the market", commented the Laboratory Manager. "It was innovative, and with the auto rotation is very easy to use."

South Devon's Laboratory Manager has since commented that "Excelsior's ease of use and maintenance makes the day easier and more controllable." All tissue processing is done overnight and workflow is very efficient. Furthermore they always achieve their targets for turnaround times.

After installing the Excelsiors in November 2004 the team at South Devon have recorded every reagent change carried out to date. They have experienced significant reduction in reagent use compared to the original processors, despite increasing daily sample throughput by almost 40 percent. It is well documented that the Excelsior can give up to 75 percent reagent savings compared to other tissue processors. In reality, South Devon has been able to go up to 14 runs before reagent rotation is required. Due to the unique alcohol quality monitoring technology, reagents are changed based on usage, block throughput and runs performed. When the change is required, alcohol 1 is simply discarded to waste and the other five alcohols automatically rotate position. Fresh alcohol is then drawn in to position 6. The simplicity of this process means a significant reduction in technologist maintenance time. It can result in several hours' labour savings per month, as well as the benefits in terms of health and safety.

The Excelsior from Thermo Scientific has long been recognised as a class leader in tissue processing, with many important features designed to give the highest tissue quality with minimum user interaction and reagent use, and optimum reliability. In 2014 the next generation tissue processor, the Excelsior AS was launched. This updated Excelsior features an intuitive touch screen interface, improved wax tank and disposable waste wax trays, additional reaction chamber heating and improved access to reagent bottles.

After over 11 years of daily use in the busy laboratory, the Excelsior ES units have proved their worth. South Devon is now looking to the future. Testament to the performance of the Excelsior ES, they recently replaced the first of the two units with a new Excelsior AS, with the second to follow in the near future. This has enabled them to maintain standardization, reduce the need for staff training on a new system and enable the rapid transfer of known protocols to the newer model without jeopardizing the quality of work.

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The Circle of Life

Tumor-activated DNA minicircles offer a potential solution for cancer detection, treatment, or both

By Michael Schubert

Most researchers looking for new methods of detecting cancer opt for endogenous biomarkers like proteins, nucleic acids or circulating tumor cells. There are obvious benefits to using a system like this – the markers are found in the bloodstream, enabling easy measuring; they offer affordable screening options; and measuring surrogate instead of true endpoints allows for smaller, faster trials with fewer ethical concerns (1). But the other side of that coin is the downsides of endogenous molecules: they often suffer from poor sensitivity and specificity because of low blood concentrations, rapid degradation, tumor heterogeneity and background expression in non-malignant tissues (2). In fact, it has been estimated that tumors may grow for as much as 12 years or longer (3) before current clinical biomarker assays can detect them, by which time they are highly likely to have metastasized. It's plain that we need better strategies for cancer detection – but if not endogenous biomarkers, then what?

At a Glance

- *Commonly used endogenous biomarker cancer tests often have low sensitivity and specificity and high background expression from normal tissue*
- *Exogenous biomarkers like engineered DNA minicircles have high sensitivity and specificity with little to no background expression*
- *The minicircles contain a tumor-activatable promoter and a reporter gene whose levels can be measured in the blood*
- *In the future, minicircles could also be engineered to contain therapeutic genes for cancer treatment*

Building an exogenous biomarker

Sanjiv Sam Gambhir and his colleagues at Stanford University, USA believe they may have a solution to that problem. They've developed an "exogenous biomarker," a DNA minicircle construct containing a tumor-specific promoter and a reporter gene that can be detected in the blood. In the case of this particular construct, the Survivin promoter (pSurv) is active in many cancers, but not in healthy adult tissues, so transcription of the human secreted embryonic alkaline phosphatase (SEAP) reporter gene is only driven in the presence of cancer. SEAP is a common reporter protein; it can be detected easily and with high sensitivity, has little to no background expression in adults, has low immunogenicity because it's a human protein, and has already seen successful use in the clinic. Pairing pSurv with SEAP in tumor-activatable minicircles (see Figure 1) offers the chance to detect cancer in patients by simply administering a systemic dose of the minicircles, allowing time for gene expression, and then measuring SEAP levels – which should only be detectable in the blood of patients with tumors.

To test the tumor-activatable minicircles, the research team used mouse models. After definitively establishing that minicircles outperform traditional plasmids in melanoma cells, they injected their constructs intratumorally into mice with melanoma xenografts, which resulted in significantly increased plasma SEAP concentrations compared with control mice – and the results lasted for up to two weeks. Unfortunately, intratumoral injections aren't always feasible in human patients, so their next step was to see whether a systemic injection of minicircles would have the same effects. Not only was the test able to discriminate easily between tumor-bearing and healthy mice, but the effects lasted well over a week, leaving a wide window of opportunity to read the test results. It was even possible to use minicircles to evaluate tumor burden. In mice with lung tumors, they measured the plasma SEAP concentrations at multiple time points and calculated the area under the curve; those values were closely correlated with the size of tumors in the lung, indicating that the new test can be used to assess not only the presence, but the extent of disease.

And the future?

Based on their mouse studies so far, the group believes the test looks very promising. Compared with endogenous biomarker tests, good sensitivity and specificity is evident – tumor-bearing mice can be distinguished from normal ones about 90 percent of the time. "In terms of sensitivity," says John Ronald, lead author on the published paper, "I'd estimate that right now we can detect a tumor about the size of a grain of rice. Now that we can do that, we're looking into making newer formulations so that we can detect smaller and smaller tumors." He explains that one of the advantages of his group's probes is that the strategy is very modular – they can test different delivery agents, different promoters, or different reporter genes. That lets them iteratively optimize the system to improve its sensitivity and specificity.

"I would say that we're within five years of testing our first-generation construct in humans," says Ronald. "Our laboratory has focused quite heavily on developing new invasive diagnostic technologies, and it generally takes us about five years to begin testing them in humans." But such technologies do require a lot of safety testing – preclinical testing, regulatory issues, and many more precautions standard for clinical trials. The first step for the Stanford scientists is to test the minicircles in breast cancer patients with known tumors. If the minicircles can detect cancer in people with

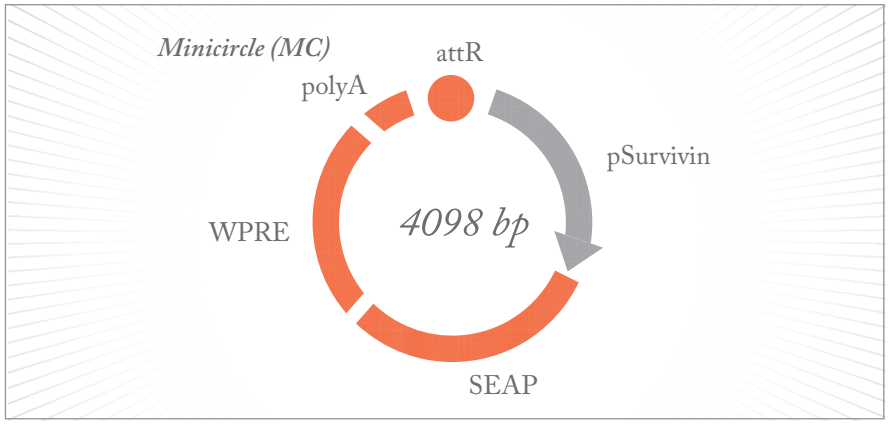

Figure 1. Vector map of the tumor-activatable minicircle, showing the promoter (pSurv), reporter protein-encoding gene (SEAP), regulatory element (WPRE), and poly(A) tail.

large tumors, then they can be tested on smaller ones to see how effective the test remains. Then, once it's fully understood how the test works in those patients, it can be attempted in people who are being monitored for tumor recurrence, and then eventually in people who don't have cancer, but are at risk – for instance, families with *BRCA1* or *BRCA2* mutations. Of course, the ultimate goal if the researchers can show safety and efficacy is to introduce this as a new blood test for screening the general population – but that's a high bar to set so early in the game.

"My vision for this test is that it will be used to detect cancer earlier than our current methods can, or to confirm cancer diagnoses made by other tests," says Ronald. "I don't expect it to be used in isolation – there will always be new types of tests, but doctors could pair our test up with a less sensitive or specific one, like an endogenous biomarker test, to get a more complete picture. Imagine giving a patient a blood test with results that indicate a possibility of cancer; with our system, instead of ordering costly and time-consuming imaging, you can simply do another blood test to confirm the diagnosis." Ultimately, he says, minicircles might be able to replace those early blood tests, but for right now, they are a tool that can be used to confirm diagnoses – which is a useful first step.

The road to better diagnostics

The Gambhir laboratory was inspired to create the system when they realized that the major problem with endogenous biomarker testing is the background expression of those biomarkers by normal tissue. One well-known example is the prostate-specific antigen (PSA) test for prostate cancer. The test fails to distinguish between people who have prostate cancer and those who just have an enlarged prostate, because normal prostate tissue also expresses that biomarker. "We asked ourselves, 'Can we reverse the problem? Can we make the cancer express something that normal tissue won't express, but that the cancer specifically expresses?' We're trying to flip the idea around by forcing the tumor to make something that's not detected at all in healthy people."

It's an idea the team came up with about three years ago. "It took a little while," Ronald reports, "because at the start, we were struggling to find the right vector. Injecting a gene-based vector is something of an unconventional approach to cancer detection, so we wanted to focus on using an appropriate vector technology. Minicircles are the minimum genetic element that is required to express a gene – and we also know that they don't integrate when you inject them systemically, so that's a good thing from a safety perspective." He admits there have certainly been challenges along the way. "I think the biggest one was figuring out how to purify the minicircles enough to get the formulations with the *in vivo* transfection agent to work. It also took a little while to engineer the right formulations, and then to detect tumors in mice, but now that the system works, we can start to make better formulations." That's the step the researchers are working on now.

Ronald does acknowledge that it's not quite all downhill from here yet. "I would say that, at the moment, there's a lot of room for improvement in our next-generation constructs. We think they won't just work for blood tests with reporter genes – minicircles can also be extended to therapeutic applications." The group is currently also exploring the possibility of creating therapeutic constructs that express both reporter and therapeutic genes, so that they can be used for a combined diagnostic and therapeutic approach. "That's the holy grail of cancer gene therapy – to express a therapeutic transgene specifically within a tumor so that healthy cells are not harmed. And it's something we might have in the next few years!"

John Ronald is the lead author on the tumor-activatable DNA minicircle study and a postdoctoral scholar at the Molecular Imaging Program at Stanford School of Medicine, California, USA.

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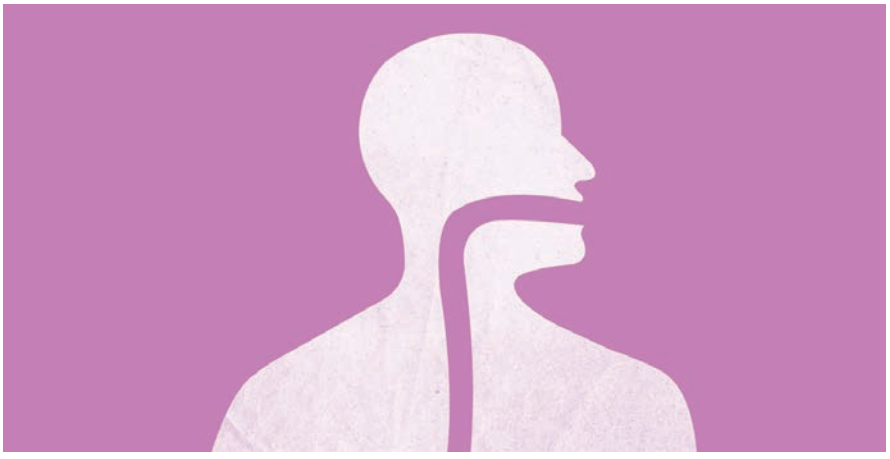
A Diagnostic That's Easy to Swallow?

A new device, the Cytosponge, could pair with biomarker testing to offer an effective, low-cost way to diagnose and stratify Barrett's esophagus

By Rebecca Fitzgerald



Much debate surrounds the question of whether or not to investigate chronic gastric reflux patients for Barrett's esophagus. On the side of investigation, Barrett's is a common premalignant lesion in esophageal adenocarcinoma, and early detection of the cancer can result in a survival rate up to six times higher than in later stages (1). But arguments against the testing include the invasiveness of endoscopy – currently the only widespread method of diagnosis – and the high cost. Given a way to overcome those obstacles, screening and diagnosis of Barrett's esophagus might become an easier, more cost-effective practice that appeals to more at-risk patients. For that reason, we've developed just such a tool – the Cytosponge, a swallowable "sponge on a string," paired with an objective laboratory test.

At a Glance

- *The current standard of care for diagnosing Barrett's esophagus is endoscopy, which is invasive and expensive*
- *Screening, especially in high-risk patients, is key to decreasing the incidence of esophageal cancer*
- *The Cytosponge, a small sponge on a string enclosed in a soluble capsule, allows sampling of the entire esophagus with a single pass*
- *Combined with biomarker testing for trefoil factor 3 (TFF3), the Cytosponge may offer a new non-endoscopic solution for Barrett's esophagus diagnosis*

Sponge on a string

Our proposed method is a non-endoscopic diagnostic test for Barrett's esophagus, which involves combining the Cytosponge with molecular biomarkers.

How does it work? The Cytosponge is a medical-grade polyester foam sphere on a string, compressed within a gelatin capsule that patients swallow while holding onto the string. Once swallowed, it takes about five minutes for the gelatin capsule to dissolve, allowing the foam sphere to expand to its full three-centimeter diameter. Then, using the string, the foam sphere is pulled from the stomach to the esophagus and out via the mouth, collecting cells along the entire length of its route. The sample is then put into a preservative – which allows it to be transported at room temperature and stored for up to several weeks – and sent to the laboratory for processing and testing.

The first step is to test for the presence of Barrett's esophagus using immunohistochemistry for trefoil factor 3 (TFF3), a protein biomarker that we've identified as being highly specific for the condition. The TFF3 test is binary; in other words, if even a single cell stains positive for this protein, it's indicative of Barrett's (2). If we see a positive stain, we next test how far along the pathway to cancer the condition has progressed. We're still in the process of perfecting the molecular analysis method, but so far, testing for mutations in the TP53 tumor suppressor gene appears to be highly specific for Barrett's dysplasia (3).

"Once swallowed, it takes about five minutes for the gelatin capsule to dissolve, allowing the foam sphere to expand to its full three-centimeter diameter."

The extent of the problem

Why is it important that a solution is

Study	Published	Type	Setting	Barrett's length	Sensitivity % (95% CI)	Specificity % (95% CI)
Pilot (6)	2007	Cohort	Secondary care	≥C1	78.0 (64.0–89.0)	94.0 (87.0–98.0)
BEST1 (7)	2010	Prospective	Primary care	≥C1	73.3 (44.9–92.2)	93.8 (91.3–95.8)
				≥C2	90.0 (55.5–99.7)	93.5 (90.9–95.5)
BEST2 (2)	2015	Case:control	Secondary care	≥C1	79.5 (75.9–82.9)	
				≥C2	83.9 (80.0–87.3)	92.4 (89.5–94.7)
				≥C3	87.2 (83.0–90.6)	
CASE1 (8)	2015	Cohort	Secondary care	≥C1 or ≥M3	95.4 (86.9–98.9)	
				≥C3	96.8 (83.7–99.5)	N/A

Table 1: Sensitivity and specificity of the Cytosponge-TFF3 test throughout the course of four studies.

found that allows the routine testing of Barrett's esophagus? The incidence of esophageal adenocarcinoma is reported to have increased six-fold in the last two decades and carries a dismal prognosis – only 13 percent of patients survive five years. This is true despite advances in neoadjuvant therapy and surgery, and has resulted in the highlighting of this cancer as a public health concern in numerous countries. Clinical guidelines currently focus on urgent referral for those with "alarm symptoms" like weight loss and swallowing difficulties, and routine referral for those with symptoms that persist despite recommended lifestyle and pharmacological management strategies. But in patients with alarm symptoms, the cancer has often already reached an advanced stage. General practice referral rates in all instances vary widely, though, and low endoscopy referral rates have been linked with poor outcomes from esophageal cancer.

Three to six percent of individuals with reflux-predominant symptoms may have Barrett's esophagus, the precursor lesion to esophageal adenocarcinoma, but less than a quarter of patients with Barrett's are diagnosed. It's estimated from some modeling studies (4) that the burden of this cancer could be as much as halved by increasing the proportion of individuals with reflux-predominant symptoms who are investigated. But that's no easy task, since dyspepsia and gastroesophageal reflux symptoms affect between five and 20 percent of the population and account for up to one-tenth of all GP consultations in the UK alone – and with recent national awareness campaigns, that number will only increase. Given the scale of the problem, and the cost of investigation, any new strategy needs to be carefully evaluated. To that end, Liam Donaldson, former Chief Medical Officer for England, highlighted the problem of esophageal cancer in his 2007 annual report (5) and recommended researching "new diagnostic techniques, including potential minimally invasive screening tests."

Endoscopic treatment of Barrett's, which progresses through dysplastic and superficially invasive stages, offers the opportunity to prevent the development of esophageal adenocarcinoma. Indeed, endoscopic treatment is now recommended by the UK National Institute for Health and Care Excellence and most international gastroenterology societies for patients with low- and high-grade dysplasia, following new evidence from randomized controlled trials. That's why we decided to develop an alternative test for Barrett's that would be suitable for primary care, acceptable to patients, and provide an accurate diagnosis at an affordable price to enable widespread use. At the same time, we've been very keen to develop molecular tests that not only diagnose Barrett's, but also risk-stratify patients, so that we can design better surveillance practices that work for patients and clinical services.

"The burden of this cancer should be as much as halved by increasing the proportion of individuals with reflux-predominant symptoms who are investigated."

Challenges in the clinic

For me, the biggest challenge is also the greatest reward – getting to work across a range of disciplines including manufacturing, public health, primary

care, health economics, biomarkers and trial design. It's surprising how long it takes for an idea to be adopted into clinical practice. We've been working on the Cytosponge for over 10 years, and even though we're making progress now, we still have a long way to go.

"In a series of four clinical studies, we've demonstrated the effectiveness of the Cytosponge."

In a series of four clinical studies, we've demonstrated the effectiveness of the Cytosponge. A feasibility study conducted in 504 patients over 11 general practices showed that it can successfully be applied to primary care, and that it's transferable to the UK National Health Service (NHS) – 27 nurses were taught to use it in a single training session, with sample processing done in an NHS pathology laboratory. It's also cost-effective compared with the current standard of care; a microsimulation model suggested a gain of 0.015 QALYs (quality of life years) and an ICER (incremental cost-effectiveness ratio) of $15,700 per QALY for Cytosponge versus endoscopic diagnosis of Barrett's esophagus followed by endoscopic treatment. It's even acceptable to patients, scoring a mean of 6 on a visual analog scale of satisfaction from 0 to 10. And as well as being practical and popular, it's had good results so far too – 2,000 patients who have been screened using the Cytosponge have shown no serious adverse events related to the device, and the test has accurately diagnosed Barrett's esophagus regardless of patient cohort or study setting (see Table 1).

Practical pathology

Unlike the multiple biopsies collected in endoscopy, the Cytosponge collects only a single sample. But owing to the nature of its collection, there is minimal sampling bias; in an experiment to investigate the clonal architecture of Barrett's esophagus, we demonstrated that in a patient with six separate clonal areas of dysplasia, all six were sampled on a single Cytosponge test (2). At the moment, the collected sample is processed to a paraffin block and sectioned for TFF3 immunohistochemistry. For risk stratification, the remaining block is then processed for DNA extraction and TP53 mutation analysis, but the optimal method of stratification is still part of our ongoing research.

Our ultimate goal is to increase the number of individuals in primary care who are tested for Barrett's esophagus without adding to the burden on endoscopy departments. The samples will still need to be processed, but we're expecting that to be part of the commercial kit so that the results can go straight to the clinician requesting the test. For patients with known Barrett's esophagus, this could be a much more efficient test than multiple biopsies, and lead to a more objective readout than the current diagnosis of dysplasia. But in the longer term, it could be rolled out as a screening service as well, if it continues to be cost-effective and meets the World Health Organization screening criteria.

As a priority we'd like to see the Cytosponge-TFF3 technology adopted as a triage test within the standard primary care clinical pathway for patients with reflux-predominant symptoms. That strategy would increase the proportion of patients diagnosed with Barrett's esophagus. In addition, we hope that it will replace the current endoscopic surveillance protocols, which are invasive, expensive, limited by sampling bias, and result in a subjective readout. A Cytosponge-sampled, biomarker-based surveillance test has the potential to address all of those limitations, so that we can effectively identify the patients at greatest risk of esophageal adenocarcinoma.

Rebecca Fitzgerald is a Medical Research Council program leader at the MRC Cancer Unit and an honorary consultant in gastroenterology and general medicine at Addenbrooke's Hospital, Cambridge, UK. She also holds a personal chair in cancer prevention at the University of Cambridge.

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Profession

Your career
Your business
Your life

From Laboratory to Leadership

How research and pathology can prepare you for a life of teaching, learning and innovating

By Michael Schubert

Not many pathologists can point to the buildings they work in and say, "I built that" – but Ulla Wewer is the exception to the rule. A researcher, a faculty dean and a Knight of the Dannebrog, Wewer is no stranger to dreaming big. For the past 30 years, she's been a key influence on the development of the biomedical sciences in Denmark, working on research at the University of Copenhagen and raising funds to build not one, but several academic centers of excellence. But from the early days of her pathology research to her appointment as dean of the University of Copenhagen's Faculty of Health Sciences, one thing has been a constant – her love for learning. "No matter where I go or what position I hold," Wewer says, "my goal is to learn everything I can and to help others do the same." Her contributions to the field range from basic research on extracellular matrix proteins and their roles in disease to designing new teaching programs, attracting innovative faculty, and building facilities in which to teach the next generation of research and medical professionals. "In looking back on my career," says Wewer, "I can see how each step led to the next, and how my enthusiasm for experimentation and discovery has given me the opportunity to look at things as small as a single protein, or as large as our wonderful worldwide community of students and researchers."

At a Glance

- *Ulla Wewer's early research revealed much of what we know today about basement membrane function and its role in disease processes*
- *Her career began with pathology. Today she is dean of the Faculty of Health and Medical Sciences at the University of Copenhagen, where she has increased enrolment, recruited world-class faculty, and raised funds to build research facilities*
- *Three new research centers have opened in less than a decade with inspiring early results, and another building will be completed next year*
- *Her focus on education continues with her work for the European Institute of Innovation and Technology, and she believes that one day, Copenhagen will serve as a model for the rest of the world as a center of scientific research and educational excellence*

From model to matrix

In the early days of her research career, Wewer's goal was to solve the mysteries of the developing cell. One of her first victories was the development of an experimental rat yolk sac tumor, which produced huge amounts of alpha-fetoprotein (imitating the visceral yolk sac) and of basement membrane material (imitating the parietal yolk sac). The latter was of great interest to many scientists because, although basement membranes are present in every tissue of the body and play key roles from tissue support to signal transmission, very little was known at that time about their composition and function. In particular, the biochemical identification of basement membrane components had been hampered by the small amounts available in normal tissues. This experimental system was an important step toward a more complete understanding of the basal lamina.

On the strength of her early work, Wewer was invited to work with Eva Engvall – the inventor of ELISA – and Erkki Ruoslahti at their La Jolla Cancer Research Foundation laboratory in California. Together, the team used Wewer's tumor model system to identify

several new proteins, including an important extracellular matrix (ECM) protein family, the laminins (1). They then studied the distribution of laminin in human breast cancer and found that neoplastic cells stained even more strongly for the protein than normal breast tissue – and that laminin staining might have potential as a method for detecting micrometastases in regional lymph nodes, whose normal cells do not stain for the protein at all (2). As there was no comparable human cell line to the rat tumor model developed by Wewer, she also prepared monoclonal antibodies to purify human laminin from placenta, allowing further research on the human laminin molecule.

Together with Wewer, the Engvall group also began work on congenital muscular dystrophy. The researchers identified the cause of one murine form as a defect in the muscle basement membranes – specifically, a mutation in the alpha 2 chain of laminin (3,4). An abnormal splice site in the Lama2 gene leads to a truncated protein that lacks the wild-type protein's ability to stabilize muscle cell membranes and contribute to ECM adhesion. Today, much more is known about the molecular causes of various forms of muscular dystrophy, and molecular pathology and genetics have become part of everyday diagnostics.

After leaving California, Wewer moved to the opposite coast of the United States to research laminin receptors at the National Institutes of Health (NIH). During her three years there, she managed to purify the laminin receptor and collaborated with several scientists to research important extracellular molecules like heparan sulfate proteoglycans. Despite not having learnt molecular biology in medical school, Wewer took courses at the NIH, learned new methods by using them in her lab, and won the prestigious Experimental Pathologist-in-Training Award from the American Society for Investigative Pathology. Her accomplishments were so impressive – and so unique, as one of the few young doctors who did long-term research in the United States – that a Danish television station came to her lab there to interview her!

"The NIH was a fantastic place for unlimited research activities for a young person," Wewer says. "'Just go ahead' seemed to be the spirit I felt, and I enjoyed collaborating with friends I still see today." The on-the-job training was particularly important to her, as it let her bring her new knowledge back to Copenhagen, where her laboratory was eventually able to clone other molecules from the extracellular environment, including tetranectin and the beta 2 chain of laminin.

Wewer's return to Copenhagen came after three years at the NIH. When she had to decide whether to stay or go back to Denmark, family and research commitments came first – and returning to the University of Copenhagen with an assistant professorship at the Institute of Pathology allowed her to spend more time with her husband and son Nicolai, while also establishing and running her own laboratory. Once set up and funded, the Wewer laboratory conducted research on ADAM12, a disintegrin and metalloprotease that functions in myogenesis, tumor cell behavior and more specifically in cell-cell and cell-matrix interactions. "I had always been keen to understand how these ECM molecules 'talked' with the cells and vice versa. Communication is key, as we all know, but how?" To find out, she worked with mouse ADAM12, cloned the human protein, and made monoclonal antibodies for testing (5,6). "Our lab in Copenhagen grew fast," she says, "and we worked hard and had fun, with collaborators and young people coming from all over world to join in with our research."

But regardless of where in the world her laboratory is located, Wewer's research career is dedicated to studying the structure and function of the ECM, cell-matrix interactions, and cell adhesion proteins – a worthwhile endeavor because of the vital role these cellular components play in diseases like cancer and muscular dystrophy.

"Her accomplishments were so impressive... that a Danish television station came to her lab there to interview her."

Building a research community – literally

"Out of the blue, Ralf Hemmingsen – a psychiatrist at the university – contacted me and said he was running for the position of dean at the faculty, and that he would like me to be his vice dean. It took him quite some time to convince me, but in the end I was ready to try something new. I had a lot of ideas for developing the faculty. I had seen so much going on in the United States – why not Copenhagen?" Wewer's period as vice dean began in 2002, but after Hemmingsen was appointed rector of the university, she became dean of the faculty, a position into which she was recently re-appointed. Her achievements since then have been impressive; through revamping recruitment, developing a strong infrastructure, and adding several new prestigious research groups, Wewer has taken the faculty to an international level. But that's not all – her dream of building research centers of excellence for her school has led to fundraising victories as well.

"I think that it's important to have common goals to successfully raise funds

Credit: CF Møller architects.

on a large scale," she says. "The foundations we've worked with have been incredibly supportive of our projects and have placed a lot of trust in us – and I feel confident saying that we all share a common vision of continued excellence and development for the University of Copenhagen and for Denmark."

One such victory has come in the form of generous donations from the Novo Nordisk Foundation (NNF), which were used to build three research centers of excellence – the NNF Center for Protein Research, the NNF Center for Basic Metabolic Research and DanStem, a stem cell research center. Wewer says the grants were awarded because the university and the NNF agreed on key strategic challenges in terms of overall themes: the recruitment of top international scientists, the use of novel technologies, and the support and development of top talents. All three research centers are now up and running and have been making strides in scientific research since their inception.

The Center for Protein Research was established in 2007 and now has about 120 people using high-end technologies to study proteins involved in genomic stability and in the biology of disease. This, the first center to open, has already released a number of high-impact publications, and scientists are now moving toward translating their results to the clinic. One of our researchers, Matthias Mann, is initiating clinical proteomics studies in diabetes research using some of the population cohorts that exist in Denmark.

The Center for Basic Metabolic Research, which opened in 2010, operates on a similar scale – with about 100 scientific staff who between them have generated over 200 worldwide collaborations, 350 peer-reviewed publications, and 5,000 citations so far. Wewer and her colleagues have equally high hopes and plans for the third center, DanStem, which has been conducting basic and translational stem cell research since 2011 and is currently supported by both the NNF and the Danish Council for Strategic Research. DanStem is focusing on developmental biology and aims – together with Rigshospitalet, a part of the Copenhagen University Hospital – to be able to transplant insulin-producing beta cells into patients with severe diabetes.

More recently, Wewer and her colleagues have been focusing on future strategies in regenerative medicine. Thus, along with Maiken Nedergaard and Steven Goldman from the University of Rochester Medical Center, they are establishing the Center for Basic and Translational Neuroscience, whose aim is to fully elucidate the role and modulation of neural stem cells and their glial derivatives. With that information, they hope to develop new strategies for cell therapeutics in the nervous system, spanning disease targets as diverse as myelin deficiencies and neurodegenerative diseases. "We want to be based on excellence in research," says Wewer, "and in the years to come, we hope to use this knowledge together with our public and private partners to contribute to better health – as we say, from molecule to society."

The newest research facility is thanks to the AP Møller Foundation, which gave the University of Copenhagen a donation to create a new research building intended to complement and advance its existing facilities. The 42,000m^2 Mærsk building (pictured) will be finished next year and will provide a new home for the Center for Basic Metabolic Research, as well as housing research into healthy aging, cardiovascular diseases, glycomics and immunology. Wewer hopes that the new facilities will encourage research across disciplines. And especially, she hopes that new initiates into the biomedical sciences will feel at home there. "I look forward to welcoming new students there in September of 2016!"

A lot has happened over the years, but Wewer is emphatic that she didn't do any of it singlehandedly – she attributes her successes to the incredible support and engagement of everybody involved in the projects. "I think one of the key routes to progress is academic leadership," she says. "Rather than establishing an environment where only a few people have power, I like to have one where all of us have fun and work together." She particularly emphasizes the good fortune of having world-class scientists in her faculty who have dedicated

all their creativity and endurance to setting and meeting the university's goals. "It's a positive spiral; the scientists are an essential part of my leadership. We're lucky to have been able to recruit top researchers not only from Denmark, but from all over the world, and I hope that more will come and be a part of our faculty. The students, too, have been instrumental in helping us to develop a modern facility that works for them. And I'd like to stress that our excellent administrative department has been vital – without their skills and support, I would never have been able to accomplish all the changes." She emphasizes that the key to her success is keeping things realistic. "Great progress is made in small steps! It's been a fantastic period of growth for the University of Copenhagen, and I hope that many future generations will enjoy dedicated and engaged work at the leading edge of international science and education."

Innovation for education

Away from her research, fundraising and administrative work as dean, Wewer also manages to dedicate time to educational initiatives. The European Institute of Innovation and Technology (EIT), a body of the European Union, has established a set of projects called Knowledge Innovation Communities (KICs). These communities bring together people from all sides of the "knowledge triangle" – namely, higher education, research and business. Wewer is the interim director of education for the EIT Health KIC, in charge of developing innovative educational programs. The KIC's main goals are to encourage students and health professionals to solve real-life problems, to bring them together with mentors and business leaders, and to educate executives on the needs of the society they serve. But it's citizens who are at the center of the knowledge triangle – Wewer believes that medical researchers need to think and act in a much more citizen-centric way. To that end, the Health KIC is establishing the EIT Health Campus, a virtual "marketplace" where learners can access educational activities that combine knowledge with best pedagogic practices. Wewer hopes that the Campus will inspire and educate through offerings ranging from short, online quizzes directed at the general population, to full EIT-accredited higher degree programs for graduate students.

"I think the clever pathologist of tomorrow may be vitally important to integrating patient information."

It all started with pathology…

Wewer feels that her own training and experience in pathology have prepared her for her current roles. "Pathology is about 'seeing' and 'reading' – and in leadership, you also need to be able to 'see' to make and execute plans, and 'read' to work in teams to make things happen." But throughout the stages of her career, the common thread has always been a desire to learn and understand as much as possible. "My experience in experimental pathology and biomedical research has kept me curious and given me countless opportunities to find out more about the world around – and within – us," she says. In particular, though, she emphasizes her love of seeing people grow and projects succeed, viewing her work with students and young researchers as a gift and an inspiration. "I find that nothing motivates me as much as the opportunity to build something new that will lead to new initiatives and new learning. Ultimately, my goal is to ensure that the University of Copenhagen can serve as a model for the rest of the world by being an inspiring place for research and education." For young pathologists, she suggests working closely not just with clinicians, but with researchers involved in – "omics" and big data. "I think the clever pathologist of tomorrow may be vitally important to integrating patient information, allowing us to come closer than ever to precision personalized medicine."

Ulla Wewer is dean of the Faculty of Health and Medical Sciences at the University of Copenhagen, Denmark, and interim director of education for the European Institute of Innovation and Technology's Health Knowledge Innovation Community.

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Stepping Out From Behind the Microscope

Sitting Down With… Blair Holladay,
CEO of the American Society of Clinical Pathology (ASCP), Chicago, Illinois, USA.

How did you get to where you are now?
Upon finishing my PhD in pathology at The Ohio State University, I began work at the Medical University of South Carolina, both as a professor teaching cancer diagnostics, and running a cytopathology and molecular diagnostics clinical trials research center focusing on translational medicine. Later, I was recruited to the ASCP as Executive Director of their Board of Certification, and appointed as Vice President of Scientific Activities. Five years after that, I interviewed and was chosen for the open position of CEO of ASCP.

Your career has seen you wearing "multiple hats"; how has this benefited you?
My work with various organizations, in different capacities, has helped me understand the ecosystem of our profession. I think it's important for us to stand together and have an organized voice. To that end, the ASCP works with multiple organizations in pathology and lab medicine to build bridges and leverage our different areas of expertise.

For example, at one point I was acting as both CEO of ASCP, and interim chief executive of the United States and Canadian Academy of Pathology (USCAP), and that was an opportunity to look at how the organizations could work together. This resulted in a Memorandum of Understanding between the USCAP and the ASCP. So I think wearing "multiple hats" has helped find ways for organizations to collaborate, such as developing residency engagement programs and enhancing our ability to create forward-thinking science education for practicing pathologists and residents.

What have been your biggest successes?
I greatly enjoy my work with the ASCP, especially being able to take important and sometimes risky steps to help our profession advance, and to address issues that are near and dear to my heart. This includes substantial international outreach – we're now working in 70 different countries, with offices around the globe, bringing education and certification to parts of the world that need it, and helping to improve standards of care while building a laboratory infrastructure that is sustainable. We work in places where many patients don't have access to diagnostic medicine, such as bringing communicable and non-communicable disease diagnostics to Sub-Saharan Africa.

Another area of passion for me is health services research, which is unusual for the ASCP, yet we've been very successful in getting grant funding from federal, industry and international sources to support this. Our aim is not just to improve diagnostics but to help educate clinicians – we need to raise awareness that pathologists and laboratory professionals are the experts in getting the right test, to the right patient, at the right time.

How big an issue is unnecessary testing?
Right now, it's huge. In the US, there's a great deal of overutilization; some reports indicate as high as 30 percent. A lot of time and money could be saved by working with clinicians to trim unnecessary test ordering and therefore costs.

The clear solution is to work with clinicians, and support pathologists and laboratory professionals serving as the consultants on which tests should be ordered. Pathology and radiology represent the majority of diagnostic medicine, and diagnostics is set to become the pivot point that determines patient treatment. I believe it's possible that in the future, diagnostic medicine will be the center where patients enter the healthcare system, and then proceed to be triaged to clinicians. So as pathologists and laboratory professionals, we have to get out from behind our microscopes and clinical testing and take on a more active, consultative role in patient care.

Another area that demands pathologist input is personalized medicine. New diagnostic assays must be validated to ensure they are adding value and evidenced-based outcomes, and not just expense. More work needs to be done to assess them – are they truly improving outcomes? If not, we shouldn't be performing these tests. It's important for our profession to play a larger role in informing patients and clinicians, and to help them make the best diagnostic choices possible.

What do you say to those who feel that pathology is just for pathologists?
You can't just divide your lab into physicians and non-physicians, in particular, as our future unfurls. We should bring diverse professionals who make up our lab teams closer together in order to meet the unique diagnostic needs of each patient. ASCP is the largest pathology and lab medicine organization in the US, with around 120,000 members; however, our most important differentiator is that we represent the entire laboratory team – not just pathologists and laboratory medicine professionals, clinical scientists, medical laboratory scientists, histologists, phlebotomists, microbiologists, and many, many more.

Instead of simply stating "pathology ought to be represented by pathologists," it's important that everybody recognize the diversity of professional leaders in our medical laboratories and encourage proactive communication and cooperation. We have always promoted this team-based approach to patient care, and I think our inclusive policies and relationships with our myriad sister organizations have been a key part of our success. Working together can only bring diagnostic improvements for our patients and enhance the future network of our professionals, a team that ultimately has the same goal—together we will ensure the best possible outcome for each patient. Every team member plays a crucial role.

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