G2 INTELLIGENCE

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Cellphones Can Detect Allergens in Food Samples

An app and a smartphone camera can help determine the presence of allergens in cooked food. Based on technology developed by researchers at the University of California, Los Angeles (UCLA) the cellphone can test for allergens in food on the spot with sensitivity similar to a laboratory test, according to a study that will appear in a forthcoming issue of *Lab on a Chip*.

The 40 gram device, called iTube, attaches to the existing camera on a cellphone to detect and optically quantify allergen contamination in food products. In this validation study the iTube accurately identified peanut traces in commercially available cookies. Sample preparation takes about 20 minutes, requiring mixing food samples with hot water, an extraction solvent, and reactive testing liquids. Following sample preparation, iTube images and automatically analyzes colorimetric assays performed in the sample and control test tubes and digitally processes them in about a second.

Lead researcher Aydogan Ozcan, Ph.D., an associate professor of electrical engineering and bioengineering at UCLA, says the iTube platform can test for a variety of additional allergens, including almonds, eggs, gluten, and hazelnuts. For more information on advances in the use of cellphone-based diagnostic technologies, please see *Inside the Diagnostics Industry* on page 5.

As Sequencing Moves Into Clinics, Tissue Sample Standards Must Evolve

As whole-genome sequencing is poised to play a larger role in clinical cancer care, a critical decision point is nearing for addressing how cancer specimens are handled, argue the authors of a viewpoint piece published Jan. 2 in the *Journal of the American Medical Association*. Standards must be established for acquiring appropriate tissue samples that can be used in routine pathologic practice to inform treatment decision in cancer genomic medicine, they say.

"Deciding how best to obtain these samples and how best to process them for whole genome or exome sequencing is a pivotal yet unresolved issue with several layers of complexity," writes co-author Eric Topol, M.D., from Scripps Translational Science Institute in La Jolla, Calif. "Pathologists currently optimize tumor sampling and processing to leverage standard diagnostic methods. However, as the new clinical applicability of genomics emerges at a fairly rapid rate, the field of pathology will arrive at the tipping point for a fundamental change in how cancer specimens are handled."

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The increased affordability of sequencing technology, coupled with the growing understanding of the heterogeneous nature of the genetic complexity of solid tumors, is causing some to question the ultimate utility of the current standard of formalin-fixed specimens in favor of frozen tissues required for whole-genome and -exome sequencing.

"Research studies using freshly frozen tumor tissue suggest an expanded capability of a 'panor-omic' assessment, which includes whole genome sequencing, RNA sequencing, and epigenomic profiling to detect methylation and histone modifications," write the authors.

This shift in specimen preference, however, poses a clinical dilemma. Medicine is evolving toward less invasive procedures like needle biopsies and aspirations, which yield smaller tissue samples. The migration toward comprehensive genomic analysis may require an extra step of sample preparation and extra tissue accordingly. The proof that larger biopsies reveal additional actionable mutations and, hence, better clinical outcomes is missing.

"Moving the pathology and oncology communities toward a new practice incorporating larger tissue samples and the routine use of frozen tissue represents a formidable but attainable change, one that will undoubtedly involve patients' preferences and consent," the authors conclude. "How quickly a new practice should emerge depends on clinical studies indicating improved patient outcomes associated with detailed genetic evaluation."

The authors note that ongoing research exploring whether free circulating plasma DNA is more representative for identifying driver mutations than the sample selected from solid tissue may reverse the need for larger tissue samples while still providing the ability to provide comprehensive genomic analysis using increasingly noninvasive means.

Pros and Cons of Routine Frozen Tissue in the Genomic Era

Pros

- No DNA cross-linking allows for easier tissue processing for sequencing.
- Critical to define intratumor heterogeneity and molecular diagnosis of key driver and passenger mutations.
- Critical to identify epigenetic changes involved in carcinogenesis.

Cons

- Requires instituting new standard of practice parallel to formalin fixation of tissue.
- Requires increased space, monitoring, and electricity for storage in freezers.
- Requires special handling during shipping to keep specimen frozen.

Source: Laura Goetz, et al., "Rebooting Cancer Tissue Handling in the Sequencing Era," JAMA, *Jan. 2, 2013*

Patients on Rituximab Not Properly Screened for HBV

Less than half of patients treated with rituximab for hematologic or oncologic (hem/onc) purposes are screened for hepatitis B virus (HBV) prior to or shortly after therapy initiation, according to a study presented at the American Society of Hematology annual meeting (Atlanta; Dec. 8 to 11). Low pretreatment screening rates demonstrate the need for continued efforts to implement evidence-based HBV screening and prophylaxis guidelines in clinical practice, the researchers say.

The researchers from UMass Memorial Medical Center (Worcester, Mass.) retrospectively analyzed data (2005 to 2011) from 103 hem/onc patients over the age of 17 years who received the monoclonal antibody rituximab (45 percent had diffuse large B-cell lymphoma, 15 percent had other high-grade lymphoma, 14 percent had follicular lymphoma, and 26 percent had another hematologic malignancy).

The researchers found that a total of 53 (51.4 percent) were screened for HBV at any point before or during treatment. Only 6.8 percent of patients were screened prior to initiation of treatment and 18.4 percent had HBV screening within 30 days of the first rituximab dose. The median time to screening in patients screened for HBV after 30 days was 196 days after rituximab initiation. Year of therapy did not affect rates of HBV screening. Of the 53 patients screened for HBV prior to or within 30 days of rituximab initiation, eight (15.1 percent) were positive for HBV infection and three were positive for HBsAg, all of whom received HBV anti-viral prophylaxis. Five patients who were negative for HBsAg were positive for HBcAb, and only one of them received anti-viral prophylaxis.

Reactivation of HBV in patients taking rituximab has been documented in both patients at high risk of reactivation (HBV surface antigen [HBVsAg] positive) and in lower-risk populations (HBVsAg negative, HBV core antibody positive), where the risk of reactivation with rituximab-based therapy has been cited as 15 percent to 20 percent. Among the 53 patients in this study who underwent HBV screening, there were no cases of HBV reactivation observed over a median follow-up time of 15.6 months.

"Altogether, there remains a critical need for standardized recommendations and consensus for screening and prophylaxis of HBV infection in patients who receive rituximab therapy," conclude the authors, led by Jayde Bednarik, Pharm.D.

Microarray Beats Karyotyping for Stillbirth Analysis

Microarray technology yields greater results in analysis of genetic abnormalities in stillbirths compared to karyotyping, largely because of the ability to analyze nonviable tissue. The improved yield can detect greater numbers of aneuploids and is more sensitive to the presence of additional pathogenic variants, according to a study published in the Dec. 6 issue of the *New England Journal of Medicine*.

The researchers from the Stillbirth Collaborative Research Network analyzed data from 532 stillbirth samples obtained from 59 different hospitals. A single-nucleotide polymorphism array (Affymetrix GenomeWide Human SNP Array 6.0) was used to detect copy-number variants of at least 500 kb in placental or fetal tissue. The microarray analysis was conducted at a single university medical center, while the karyotype analysis was conducted in multiple university-affiliated laboratories.

Microarray analysis yielded significantly more results than karyotyping (87.4 percent versus 70.5 percent) and provided better detection of genetic abnormalities (aneuploidy or pathogenic copy-number variants) than karyotyping (8.3 percent versus 5.8 percent). Microarray analysis provided a relative increase in the diagnosis of genetic abnormalities of 41.9 percent in all stillbirths, 34.5 percent in antepartum stillbirths, and 53.8 percent in stillbirths with anomalies, compared with karyotyping.

"Microarray analysis is more likely than karyotype analysis to provide a genetic diagnosis, primarily because of its success with nonviable tissue, and is especially valuable in analyses of stillbirths with congenital anomalies or in cases in which karyotype results

cannot be obtained," say the authors, led by Uma M. Reddy, M.D., from the National Institutes of Health in Bethesda, Md.

Variants detected that were not identified in three reference databases were classified into three groups: probably benign, clinical significance unknown, or pathogenic. Some of the pathogenic variants that were detected on microarray analysis may represent unbalanced translocations, which can be missed by karyotyping.

Only microarray analysis detected three copy-number variants in three stillbirths at chromosome 22q11.2, a region disrupted in the DiGeorge syndrome. A recurrent variant of unknown significance in a telomeric region of chromosome 19p13.3 (ranging in size from 632 kb to 930 kb) was seen in eight stillbirths, a region known to contain multiple benign variants as well as five loci associated with disease, but not with stillbirth or developmental disorders.

When factoring variants of unknown significance into the comparison of karyotyping versus microarray technology, there was an even greater significant detection of abnormalities with microarray technology (13 percent versus 5.8 percent). Of the 157 stillbirths for which karyotyping failed to yield a definitive result, 79.6 percent yielded a definitive microarray result, of which 5.7 percent were abnormal. G2

Sequencing IDs Therapy-Sensitive HER2 Mutations

Next-generation sequencing-based testing can identify human epidermal growth factor receptor 2 (HER2) mutations in breast cancer not detected using standard HER2 testing. Many of these somatic mutations drive tumor growth and may respond to HER2-targeted drugs typically reserved for HER2-positive or overamplifying patients, say the authors of a study published online Dec. 7 in *Cancer Discovery* in conjunction with a presentation at the San Antonio Breast Cancer Symposium.

"HER2 somatic mutation is an alternative mechanism to activate HER2 in breast cancer and they validate HER2 somatic mutations as drug targets for breast cancer treatment," write the researchers, led by Ron Bose, M.D., Ph.D., from Washington University (St. Louis).

Using DNA samples from eight breast cancer sequencing studies, the researchers identified and functionally characterized 13 HER2 somatic mutations in 25 HER2 gene amplification-negative breast cancer patients. Seven of the 13 mutations drove cancer growth with most of these mutations responding well to the anti-HER2 drugs lapatinib and trastuzumab or to neratinib, a newer anti-HER2 drug in clinical trials. Sounding caution, however, the researchers acknowledge that some of the mutations did not drive tumor growth and would likely not respond to HER2-targeting drugs.

"If we can identify mutations that we can act on, that information will help us better guide treatment," said Bose in a statement. "In this case, we don't even have to develop new drugs against HER2 mutations. It's just a matter of finding the patients."

The researchers estimate that undetected HER2 mutations—rather than the HER2 amplification—may be driving tumor growth in 1.5 percent to 2 percent of all breast cancer cases, or nearly 4,000 patients annually. As a result of these findings, the researchers have launched a phase II clinical trial to test whether HER2-negative, stage IV breast cancer patients will benefit from anti-HER2 drugs. G2

Illumina Expanding Presence Into Diagnostics

Greg Heath, Ph.D.

Over the last few years, next-generation sequencing company Illumina (San Diego) has made a concerted effort to diversify. With a growing pipeline of diagnostics products and two strategic acquisitions (in 2012 BlueGnome, a maker of cytogenetics and in vitro fertilization screening products and this January noninvasive prenatal diagnostics maker Verinata Health), the company is moving away from being strictly an instrument manufacturer, even while its core business remains strong with 2012 revenue expected to be between $1.134 billion and $1.144 billion.

Gregory Heath, Ph.D., general manager of diagnostics at Illumina, recently spoke to *DTTR* about the company's strategic vision for its diagnostics segment and plans for the division's growth.

Please tell us about Illumina's strategic decision to diversify and enter the diagnostics market.

A little over four years ago Illumina decided they were quite successful in the life science business so they wanted to diversify into other areas, and molecular diagnostics is one of the biggest areas.

I joined from Roche, and we started building up the diagnostics business. One of the first things we did was look at strategy and selected a couple of areas where we thought our technology would really be a good fit. Our technologies are high-density microarrays and NGS [next-generation sequencing]. The four areas clinically we have settled on are genetics, with a particular emphasis on reproductive genetics, cancer, infectious diseases, and transplantation.

What does your pipeline of diagnostic products look like in each of these selected clinical areas?

For the short term the areas we are focusing on are genetic disease and cancer. Within reproductive genetics we recently acquired a company called BlueGnome, which works in the IVF space with preimplantation genetic diagnosis and preimplantation genetic screening. We have a cystic fibrosis test in development, which we are getting ready to submit to the U.S. Food and Drug Administration, and both Illumina and BlueGnome have cytogenetics products that we plan to submit after that. That is an array-based platform, not based on NGS, but it has certain characteristics that are well suited to the cytogenetics market.

Within the cancer space we have focused on a couple of things in the short term. We are working with a number of labs on somatic mutations panels. We have also been in discussion with a number of companies for companion diagnostics. We think cancer is a big opportunity in the longer term.

In infectious diseases, the technology has really moved fast but it probably needs to be a little cheaper and a little faster. But the coverage is better than what we typically see with hotspot analysis that is done today. . . . For HIV, there I think we have a different approach where the technology will allow you to circumvent hotspot testing. If somebody's viral load is rising because the virus has mutated and you want to know exactly what that mutation is, you can sequence the whole viral genome. Those applications will come as the technology gets cheaper and faster.

The last area we see as an opportunity is transplantation, particularly HLA-typing, looking at bone marrow transplants as well as solid organ. There again, I think we need to be a little cheaper on the blood marrow side and a little faster on the solid organ side, but that is coming quickly.

Will the diagnostics division pursue additional acquisitions to drive growth?

The business is young and growing. We see growth coming from both [internal development and acquisitions]. If we see a good acquisition that makes sense for the company we are always interested. It has to be a good fit with our technology and in our areas of interest (genetic disease, cancer, infectious disease, and transplantation) where we believe sequencing will play a significant role going forward. BlueGnome made a lot of sense because they had a good software platform, BlueFuse Software, and their IVF side of the business was growing rapidly and it was very compelling.

What clinical applications are most likely to routinely adopt NGS-based testing in the near term?

Cancer is an area where we have great alignment with the performance characteristics of the platform and the need in the market. For example, by deep sequencing you can tease out the heterogeneity of the tumors. In our CLIA lab when we look at applications, what we tend to see is people who are cancer cases at the end of standard of care—that they've exhausted their options and are looking for some alternative, maybe even an experimental protocol they can be placed on. We can add value there.

In genetics we see pediatric and adult cases where typically people will go on this diagnostic odyssey where physicians will test them for one thing—kind of piecemeal. When someone comes to our lab they get their whole genome sequenced and the physicians can hypothesis test in silico. They can look at that first gene, immediately discover they were wrong, and move on to the second gene without sending the patient back for additional samples or testing.

How will clinical use of NGS-based testing evolve in the next five years?

Illumina has a good vantage point because we have such a large footprint on the research side. Everything that is going to wind up in the clinic is going to start with research. Something like 90 percent of all bases sequenced are done on Illumina platforms. We partner with a lot of people and we can see which changes are emerging.

Cancer will be an area that in the not-too-distant future there will be many more applications. Pharma is investing heavily in oncology. There are a limited number of drugs available for any particular cancer today, and there are only a handful of genes that currently drive those treatment decisions. I think with whole-genome sequencing

Illumina Acquires Verinata Health*

In a move that will further bolster the company's position in reproductive diagnostics, Illumina announced on Jan. 7 the $350 million acquisition of Verinata Health (Redwood City, Calif.), the maker of the verifi noninvasive prenatal test for chromosomal abnormalities, including trisomies 21, 18, and 13. An additional $100 million in milestone payments is expected through 2015. Illumina is financing the deal primarily with cash on hand and expects the acquisition to become accretive in 2014. The company says that since noninvasive prenatal testing is one of the most rapidly growing applications for next-generation sequencing, Illumina is ideally suited to be a player in this space. The domestic market for noninvasive prenatal testing will reach $1.5 million to $2 million within five years, the company says.

"The synergies between Verinata's and Illumina's capabilities, combined with the expertise in reproductive health gained from the acquisition of BlueGnome, enable Illumina to provide a compelling portfolio of offerings across the spectrum of reproductive health," said Heath in a statement.

**The acquisition was announced following* DTTR's *conversation with Greg Heath, Ph.D.*

we are going to see many, many more markers come to the forefront and, hopefully, many more therapeutics. As the therapeutics proliferate the number of choices physicians have to make goes up, and to inform those choices we think NGS will play a role.

Genetic disease testing will continue to proliferate. This is the right technology to do genetic diagnosis, particularly around reproductive genetics. We saw the emergence of noninvasive prenatal testing recently. We are seeing a lot of people on the research front working on immunosequencing, microbiome, and methylation, primarily on arrays. All of those will emerge, and we will see short-term breakthroughs in genetics and cancer and along the way technically infectious disease and transplantation will be possible, but [the technology] probably needs to get a little cheaper and faster to be economically feasible. . . . I see NGS as being applied broadly in the next three to five years.

Reimbursement and regulatory issues are frequently cited as challenges diagnostics manufacturers must overcome for successful market penetration. How are these issues affecting NGS-testing?

Health care costs are going up. But our costs are going down so we think we are creating a lot of value for the health care system. Within reimbursement I think it depends a little bit on whether there are existing codes and this is a technological substitution for an existing approach, in which case reimbursement may be in place already. Or, if this is a new-to-world test, reimbursement has to be established. We see out-of-pocket pay for these new-to-the-world tests.

For a pharmacogenomic example, if somebody has breast cancer the choice may be aromatase inhibitors, which cost $30,000 for a course of therapy, versus tamoxifen, which may be $7,000 for a course of therapy. If you happen to be a poor metabolizer for tamoxifen, you won't be able to convert it to an active state. You will be on chemotherapy, have all the side effects, but none of the benefits. If I were a physician I would want to know which patients should be on the aromatase inhibitors. If I were a patient, I would definitely want to know that, and if I were a payer there is an economic incentive to look at it. You could easily cover the cost of even a several-thousand-dollar test with that price difference.

Regulatory issues will be a challenge, but there are nuances there too. The FDA wants to be considered innovative and bring innovative technology through, but part of the challenge with NGS is, what's the gold standard you compare to? If you compare it to Sanger sequencing, it is almost like comparing your high-definition TV to a black and white TV and saying it is just as good. It is not really a good comparison. There is also the issue that with high resolution you can see a lot of things that are real changes. Analytically you know the mutations are there, but they may lack clinical significance. So what do you do with those? Clinical science will take some time to catch up and that is what drives physician adoption. But for a lot of physicians today, if their patient is out of options, they may look at NGS for those cases.

Illumina by the Numbers*

- Founded: 1998
- Initial Public Offering: 2000
- Number of Employees: 2,400 globally
- Revenue (2011): $1.06 billion
- 10-year CAGR: 83%
- Published studies based on Illumina technology: 3,484-plus
- Percentage of all sequencing data produced on Illumina systems: 90%

**Illumina does not break down financial reporting for its diagnostics segment at this time.*

Interest High in Cellphone-Based Diagnostics for Field Testing

Mobile health is changing health care delivery worldwide. Now, emerging cellphone-based diagnostic tests are altering the future for how laboratory testing will be conducted, particularly in remote areas. Cellphones are proving capable of imaging, sensing, processing, and communicating health-related data nearly instantaneously in field settings.

It is estimated that there are more than 5 billion cellphone users globally and that 80 percent of the world's population lives within range of a cellphone tower. In many underserved areas the wireless telecommunications infrastructure exceeds the reach of the health care infrastructure. This wireless infrastructure combined with cameras and digital components embedded in smartphones and cloud-based computing marks a powerful new front to fight global disease and helps bridge the gaps in access to health care.

Applications for the cellphone-based diagnostic technology are near limitless with validation efforts already progressing for technologies using smartphones for digital microscopy and enabling rapid, qualitative immunoassay screening for infectious diseases, water quality, and food safety even in resource-limited settings. Experts say that products linking in vitro diagnostics products to cellular technology may be a crucial component in how the clinical diagnostics industry penetrates emerging markets in developing nations and will further propel the migration of clinical lab testing away from a central laboratory. Early evidence indicates that governmental agencies and nongovernmental organizations are both interested in these new, handheld technologies. Additionally tying the point-of-care test results into cloud-based monitoring can aid public health workers in identifying and managing disease outbreaks.

"By applying technology you can leverage more limited human resources and can create more consistent, controllable standards of care and you can magnify the capabilities of health care workers."
—Gaetano Borriello, Ph.D.

"An aspect of public health when working in these areas is to take advantage of the resources you do have," explains Gaetano Borriello, Ph.D., the Jerre D. Noe professor of computer science and engineering at the University of Washington. "In Tanzania there is one physician for 50,000 people. In Seattle there is one for 300 people. That is like all of Seattle having only 20 doctors. By applying technology you can leverage more limited human resources and can create more consistent, controllable standards of care and you can magnify the capabilities of health care workers."

Unlike in laboratories located in resource-rich settings, microscopes and cytometers may be unavailable in remote areas in developing countries, as are the skilled technicians necessary to run the tests and interpret results. Diagnoses in these underserved areas are further hampered by the frequent inability of patients to make long journeys, often by foot, to return to clinics to obtain test results and medications, if necessary.

The advent of rapid diagnostic testing using lateral flow technology has made disease screening quicker and simpler allowing for testing in decentralized and often more convenient locations. Rapid diagnostic tests are commercially available for a wide variety of infectious diseases including HIV, malaria, tuberculosis, and syphilis.

"Conventional rapid diagnostic tests are currently read manually, by eye, which is prone to error, especially if various different types of tests are being used by the health care worker," said Aydogan Ozcan, Ph.D., a professor of electrical engineering and bioengineering at University of California, Los Angeles, whose lab has developed and is working toward commercialization of both microscopes and rapid diagnostic testing readers that clip onto cellphones.

How It Works

Ozcan has developed a universal digital reader for all rapid diagnostic tests, which eliminates the need for manual decisionmaking and test interpretation. The reader, which weighs about 65 grams, clips onto a cellphone and includes an inexpensive lens, three LED arrays, and two AAA batteries. Improving upon some existing digital test readers, which are limited to test strips from a particular manufacturer, this platform is manufacturer-agnostic and can read nearly every type of rapid diagnostic test.

A strip is inserted and converted into a digital image (within less than 0.2 seconds per image) using the cellphone's existing camera unit and an app. The platform then rapidly reads the digitized test image to determine both if the test is valid and whether the result is positive or negative. Ozcan says the automation eliminates the potential for human errors, especially if the technician is administering multiple test types.

Mobile Assay's Workflow

Source: Mobile Assay

The platform wirelessly transmits the test results to a global server, which processes them, stores them, and, using Google Maps, creates a map charting the spread of diseases both geographically and over time. Additionally, since the color changes in test strips don't last more than a few hours in the field, the ability to store the digitized image indefinitely provides an added benefit. Ozcan says the platform has been tested using malaria, tuberculosis, and HIV both on Android-based smartphones and an iPhone.

There are currently two strategies being employed in the development of cellphone-based diagnostics platforms. The first, as is the case with Ozcan's technology, employs an attachment that will require U.S. Food and Drug Administration (FDA) approval as a medical device. Another strategy utilizes the cellphone's technology strictly to transfer digital information to a cloud-based platform, where the processing and analysis occurs. While the FDA has yet to release definitive guidelines for approval of mobile-based diagnostics, Mobile Assay (Boulder, Colo.) is betting this approach will be quicker to be cleared through the FDA with validation required only for the server and software component, rather than cellphone add-on components.

"If you make the phone anything but a conduit of information by using an add-on product, then all of a sudden there will be a whole lot more validation required," says Warren Mauter, chief commercialization officer for Mobile Assay. "If you define a mobile product as a transporter of information and everything happens on a validated server and validated software, it will likely be a more straightforward process. Otherwise, what phone, what [operating system] version, each variable has to be addressed."

Additionally, Mauter believes that eliminating add-on units also broadens the potential market as analysis can be conducted with any phone with a camera. Mobile Assay's technology employs similar components—cellphones, commercially available test strips, geographical tagging, and cloud computing. However, its system relies upon Mobile Image Ratiometry (MIR; developed by University of Colorado associate professor Don Cooper, Ph.D., chief science officer of Mobile Assay). MIR is a novel software algorithm that analyzes images and can precisely quantify the level of infection from test strips that previously only afforded qualitative analysis.

"If you define a mobile product as a transporter of information and everything happens on a validated server and validated software, it will likely be a more straightforward [regulatory] process."
—Warren Mauter, Mobile Assay

Mobile Assay recently won a $100,000 Grand Challenges Exploration grant awarded by the Bill & Melinda Gates Foundation to fund a project titled "Lab on Mobile Device Platform for Seed Testing." The company will initially target the fungus Botrytis—which can devastate crops like yams, potatoes, wheat, soybeans, onions, and sorghum around the world—and aflotoxins produced by Aspergillis fungi, which can contaminate seeds during storage and are among the most carcinogenic substances known. Cooper said the company's MIR imaging technology can increase the sensitivity of test strips for Botrytis and aflotoxins by a factor of 100.

Mauter says Mobile Assay is using a business model that relies on partnering with strategic partners with existing manufacturing or distribution channels to offer a premium service allowing for real-time quantification for commercial and clinical uses including applying the technology to quality control tracking at dairy farms. In addition to agricultural and global health applications, a number of health tests ranging from high cholesterol to abnormal thyroid-stimulating hormone levels could be conducted at home using specific test strips, with the data made available immediately to their health care providers over the Internet, Cooper suggests.

Toward Commercialization

"The diagnostics market in the United States is much better understood. We know better what to expect, like a black box with input and output," Ozcan tells *DTTR*. "The diagnostics market for global health is less well understood . . . and harder to make a profit. [We want to] first enter the developed world with a disruptive innovation and take market share, then we can take more risk, recycling the technology and some of the know-how."

Commercialization of these technologies is in the earliest of stages. Some of these companies spun off from university research have been successful in raising funds necessary for validation studies. Here is a sampling of some of the companies working on cellphone-based diagnostic products:

- **Holomic** (Los Angeles), an optics company that was established to commercialize Ozcan's technologies, is working on bringing to market a portfolio of microscopes and rapid diagnostic test readers. The company raised $2.5 million in seed funding from a strategic investor as well as $383,000 from a National Institutes of Health Small Business Innovation Research (SBIR) grant. The first product that will reach the market (expected in early 2013, Ozcan says) is based on LUCAS technology (Lensfree Ultra Wide-Field Cell Monitoring Array Platform based on Shadow Imaging). The lens-free, holographic microscope can attach to a camera unit of a cellphone. Slide samples are illuminated by a simple light-emitting diode (LED). This light is then scattered from biological sample, creating a hologram that can be used to mathematically reconstruct the microscopic image of the object on the detector array of the cellphone without the use of lenses. These holographic signatures are rapidly digitally processed and can be used to image blood cells, waterborne parasites (Giardia lamblia), or infectious agents (E. coli).

- **Lifelens**, a joint venture of researchers from several universities, including Wilson To, a Ph.D. student at University of California, Davis, at the time of the project's inception, has developed a cellphone-based malaria diagnostic tool. The team has received funding from Microsoft Corp. as well as a $75,000 Microsoft Imagine Cup grant. The diagnostic system runs on a Windows Phone 7 operating system and uses a special lens attachment (350x zoom) to take a picture of a standard stained blood smear and runs an automated computer vision analysis to find any parasites present in the blood and determine the density (and progression) of the infection. Results take about two minutes. The geolocation-stamped information can then be uploaded into a network cloud to provide real-time worldwide data about malaria outbreaks. The group is looking to improve upon the power and cost of the $50 lens and says they are currently testing lenses of different materials that have magnification capabilities of 100x to 600x. The Lifelens team says the system has a 94 percent accuracy and a false positive rate of 10 percent to 15 percent, although results have not yet been published. Clinical trials are expected to begin by the end of summer 2013.

Holomic's Lensfree Cellphone Microscope

Lensfree Cellphone Microscope

Source: Ozcan Research Group at UCLA

- **CellScope** (Berkeley, Calif.) spun out of research developed by Daniel Fletcher, Ph.D., a professor at the University of California Berkley, is based on technology that allows the camera of a standard cellphone to be turned into a diagnostic-quality microscope with a magnification of 5x to 60x, enabling the visualization of samples including blood smears and urine samples, followed by capture, organization, and transmission of images. The company envisions that most routine microscopy can be done on CellScope, including applications for malaria, sickle cell, and tuberculosis in global health as well as application of the platform for remote ear, throat, and skin exams in the United States. The startup received funding through Rock Health in 2011, a $1 million seed fund investment from Khosla Ventures to bring to market its otoscope smartphone attachment for remote diagnosis of ear infections. The patent-pending optical attachment is currently being tested by physicians in the Bay area.

High-Throughput Screening IDs Effective Drug Combos

Using drug combinatorial screening can identify effective combinations for melanomas with genetic variants, including BRAF and RAS mutations that confer either primary or secondary resistance to known therapies. According to a study published in the January issue of *Cancer Discovery*, the high-throughput drug screening method identified previously unrecognized patterns of drug interaction with the potential for clinical efficacy in treating defined subgroups of melanoma. Such a screening approach may be applicable to other cancers.

Using an array of small-molecule inhibitors on early-passage melanoma cultures, the researchers applied a systematic combinatorial high-throughput drug screening (cHTS) approach to evaluate the selectivity of drugs, alone and in pairs, and in the context of BRAF- or RAS-activating mutations (affecting 40 percent and 20 percent of human melanomas, respectively) in the hopes that more defined genotype-selective patterns would yield higher efficacies, thus combatting the problem of limited responses to single agents.

"Some patients who have a specific cancer-driving genetic mutation never respond to the matching drug, while nearly all those who initially respond eventually become resistant to the effects of the drug," says co-author David Stern, Ph.D., from Yale University School of Medicine in New Haven, Conn. "There is a great need for drugs to treat cancers driven by RAS. RAS proteins are inappropriately active in up to a third of all human cancers, including melanoma and lung and pancreatic cancers."

Single agent analyses of 150 drugs were individually conducted and then cHTS tests were performed on 40 combinations. Genotype-selective combinations were determined using data for drug pairings that yielded an average minimum of 15 percent growth inhibition exclusively in a genotypic group. The researchers found melanoma cell lines driven by BRAF and RAS were sensitive to different novel combinations of drugs including the pairing of statins with cyclin-dependent kinase inhibitors.

"Our high-throughput screening approach is applicable to other types of cancer, including lung and pancreatic cancer," Stern says. "A major challenge is in picking the appropriate agents for combination screening, since with multiple doses per agent, the scale of a screen needed for all combinations grows rapidly. This requires careful evaluation of single agents and analytical methods for choosing the best candidates for follow-up in combinations." G2

Inflammatory Markers Associated With Depression

There is an association between elevated plasma levels of C-reactive protein (CRP) and an increased risk for psychological distress and depression in the general population, according to a study published online Dec. 24 in the *Archives of General Psychiatry*.

CRP is a commonly used marker of inflammatory disease when CRP levels exceed 10 mg/L. Previous studies, though, had yielded conflicting results regarding the strength of the association between CRP levels and psychological distress and depression, for which an inflammatory-related pathogenic mechanism is not understood.

For the study the researchers examined data on 73,131 men and women (aged 20 to 100 years) derived from two general population cohorts (the Copenhagen General Population and the Copenhagen City Heart studies). They analyzed CRP levels in clinically relevant categories of psychological distress and depression. Psychological distress was measured using self-reported symptoms. Depression was ascertained using self-reports of anti-depressant uses, national prescription registries, and hospital discharge diagnoses of depression.

The researchers found that CRP levels were associated with increasing risk for the self-reported symptoms of psychological distress of "not accomplishing much" and "wanting to give up." Increasing CRP levels were also associated with a significantly increasing risk for self-reported use of anti-depressants as well as for increasing odds of using of prescription anti-depressants for at least six months, and an increasing risk for hospitalization due to depression. Contrary to previous studies, these associations did not disappear when adjusting for body mass index and chronic disease status.

"The results also support the initiation of intervention studies to examine whether adding anti-inflammatory drugs to antidepressants for treatment of depression will improve outcome," wrote lead author Marie Kim Wium-Andersen, M.D., from University of Copenhagen in Denmark.

Telome Health Set to Launch Saliva-Based Telomere-Based Health Assessment

Telome Health (Menlo Park, Calif.) plans to launch a saliva-based test of telomere length, protective caps at the ends of chromosomes, to help assess health status, disease and mortality risk, and response to specific therapies. Data presented at the American Society of Human Genetics (ASHG) annual meeting (San Francisco; Nov. 6-10) demonstrates the prognostic utility of the test.

"Telomeres are one of the few parts of the genome that can be changed by lifestyle choices, and hence telomere length measurements can provide valuable feedback on one's disease risks and, potentially, the effects of lifestyle changes," said Telome Health co-founder Elizabeth Blackburn, Ph.D., who received the Nobel Prize for her telomere discoveries. "The data from the saliva-based testing in the large Kaiser-UCSF research study is an exciting step forward in the field of telomere science, as it helps to advance the use of telomere testing into regular clinical practice."

Telome Health acquired the worldwide rights to the quantitative polymerase chain reaction-based telomere test from the University of Utah in 2010. This new study also confirmed that clinical associations with telomere length remain preserved when using saliva specimens rather than blood.

The TeloTest diagnostic test measures the average telomere length, which is known to shorten with age. But the rate of shortening can be accelerated because of stressors—a variety of biochemical, viral, or mechanical stressors. The company believes using telomere length as a "health barometer" can be useful in personalizing clinical care and health maintenance.

The Kaiser Permanente-University of California San Francisco study linked subjects' medical records with telomere testing results in 100,000 participants. The study showed that individuals who had the shortest telomeres had a significantly increased risk of death over the three-year follow-up period, even after adjusting for lifestyle factors. Smoking, heavy alcohol consumption, lower education, and poor environments were associated with short telomeres, while moderate exercise was associated with longer telomeres.

The Kaiser Permanente-UCSF Genetic Epidemiology Research GWAS Study

In a separate study presented at the ASHG meeting, the Kaiser-UCSF group presented results of a genomewide association study conducted on the 675,000 SNPs assayed for each of the 100,000 participants—a scale usually reserved for meta-analysis.

The researchers identified 63 significant genomic regions including revalidation of the importance of two known telomere maintenance genes (OBFC1 and TERC genes). Additionally, the group demonstrated that 14 of the 63 identified regions showed genome-wide significance in at least one other major race or ethnicity group besides the 70,000 white subjects used in the primary analysis. Two of these SNPs (on chromosomes 1 and 12) that encode amino acid substitutions, showed "extreme significance" and another eight loci have P values less than 10^{-20}. While these loci have yet to be determined functions in telomere maintenance with age, the researchers say that the sizable number of new highly significant locations will propel further research into the role of telomeres in health and disease.

"If you understand your baseline telomere length you can change your lifestyle or drug regime and use telomere length to monitor it," Calvin Harley, Ph.D., Telomere Health's chief scientific officer, tells *DTTR*. "We also know that rate of loss of telomere length is important and we can monitor over time."

Harley explains that the company plans a three-stage commercial rollout. In the first stage, the test will be offered as a measurement for wellness, with a soft launch expected first quarter. The lab-developed test will be run in the company's soon to be licensed Clinical Laboratory Improvement Amendments-waived laboratory with a hard launch to follow in the second quarter. Physician orders are required and the test will initially require out-of-pocket payment, anticipated primarily through concierge physicians and corporate wellness groups. In the second phase, expected in 2014, the test will incorporate telomere length as a clinical diagnostic factor including a clinical diagnostic threshold for which doctors can take "more aggressive drug intervention," Harley says. The third phase, which will be two years to five years away, would be to get TeloTest approved through the U.S. Food and Drug Administration as a companion diagnostic to monitor therapeutic effect.

Small, Strategic Acquisitions Prevail in Closing Days of 2012

In the closing days of 2012 economic uncertainties prevailed, including matters that would directly impact the financial future of the diagnostics industry. The fate of the research and development tax credit and the budget health of the National Institutes of Health weighed heavily on medtech investors. Other economic stresses, like the impending 2.3 percent excise tax on the medical device industry and the 2013 increase in federal capital gains tax rates, loomed.

Overall, mergers and acquisitions in the life sciences industry were down, experts say around 33 percent compared to 2011, and given investors' reservations there were no

blockbuster acquisitions in the waning days of 2012. Instead the diagnostics industry saw a series of small transactions primarily driven by methodical moves—calculated divestitures, solidification of intellectual property (IP) portfolios, and strategic enhancements to existing product lines.

- **Dec. 31**—Access Genetics (Eden Prairie, Minn.) acquired Quest Diagnostics' (Madison, N.J.) OralDNA labs. The salivary diagnostics unit was divested as an ongoing part of Quest's reorganization to concentrate on services for physicians and hospitals, the company said. OralDNA serviced the dental industry. Terms of the deal were not disclosed.

- **Dec. 28**—Miami-based OPKO Health made two December acquisitions to expedite its commercial launch of its 4Kscore prostate cancer test both in the United States and abroad. At the end of the month OPKO Health acquired Silcon Comércio (Brazil) to more rapidly establish a presence in the high-growth Brazilian market. Just two weeks earlier OPKO Health purchased Prost-Data (a Nashville-based Clinical Laboratory Improvement Amendments-waived laboratory doing business as OURLab). OURLab has 18 phlebotomy sites nationally and an experienced sales force calling primarily on urologists, which is expected to accelerate OPKO Health's domestic commercialization strategy.

- **Dec. 26**—Amarantus BioSciences (Sunnyvale, Calif.) purchased all of the IP assets from bankrupt Power 3 Medical Products. Included in the sale were five patents for the diagnosis of neurodegenerative diseases, 20 pending patent applications (for biomarkers and assays related to Parkinson's disease, amyotrophic lateral sclerosis, Alzheimer's disease, chronic myelogenous leukemia, and breast cancer), and all supporting data sets. The assets were acquired for $40,000 and will aid in the commercialization of the NuroPro Parkinson's disease test (previously licensed from Power 3) as well as enhance the diagnostic pipeline for Alzheimer's-related products.

- **Dec. 21**—Dako (an Agilent Technologies company; Denmark) acquired the IP behind System Dynamics' (Santa Barbara, Calif.) connectivity software. The two companies have worked together for 12 years on software used in Dako's Autostainer and Artisan cancer diagnostics instruments. Terms of the deal were not disclosed.

- **Dec. 16**—Bio-Rad Laboratories (Hercules, Calif.) acquired antibody manufacturer AbD Serotec (a division of MorphoSys AG; United Kingdom) for 53 million euros in cash. The transaction is expected to close in January. Bio-Rad says the acquisition gives the company access to a comprehensive catalog of antibodies allowing it to offer total assay solutions that can be validated on its research platforms for western blotting, multiplex protein expression, ELISA, and cell sorting.

- **Dec. 13**—Antibody-based tool company Rockland Immunochemicals (Gilbertsville, Pa.) bought the TrueBlot IP/Western blot product line from eBioscience, (an Affymetrix company; San Diego). The TrueBlot product line will expand Rockland's Western blot workflow offerings. eBioscience divested the Western blot line to focus on flow cytometry, immunoassay, and immunohistochemistry applications in the immunology and oncology fields.

G2 Insider

Are Serial Malaria Tests Still Necessary With Rapid Diagnostics? . . . Traditionally to rule out a diagnosis of malaria requires three negative blood films. But in the era of routine rapid diagnostic testing, some are questioning the need for continued serial testing. A new study, published online Dec. 3 in the *American Journal of Tropical Medicine and Hygiene*, shows that for patients not taking anti-malarial therapy nearly all malaria diagnosis are made with the initial blood film and rapid diagnostic test, possibly obviating the need to continue serial testing.

Australian researchers examined 255 cases of malaria diagnosed from 1999 to 2010 at three laboratories in a nonendemic area. Standard operating procedures at the three labs require that all clinical requests for thick and thin blood films for malaria also have a reflex rapid diagnostic testing performed (BinaxNOW immunochromatographic test). Plasmodium falciparum malaria was diagnosed on the first diagnostic set in all but one case (99 percent), while initial tests detected more than 97 percent of cases for non–P. falciparum malaria. Nine cases of malaria (3.5 percent) had negative results for the initial blood film and rapid diagnostic test but were diagnosed on the second film. The researchers say that each of the missed cases was atypical with four of the patients having received anti-malarial medication, which is known to lower parasite density and modify the appearance of parasites on blood films.

Despite research showing that rapid diagnostic tests have excellent test characteristics to exclude malaria, serial testing is still recommended. Serial testing requires laborious sample preparation and examination of blood films that hampers laboratory workflow, hospital efficiency, and increases patient wait times and costs. With the vast majority of malaria diagnoses made on the first set of tests, continuing serial testing may be unnecessary.

"Our findings suggest that for patients with imported malaria who have not been exposed to anti-malarial drugs, the diagnosis is likely to be made on the first set of thick and thin blood films combined with rapid diagnostic tests," write the authors, led by Janet Pasricha, from Royal Melbourne Hospital. "Larger, prospective studies are now required to assess the safety of this approach."

Company References

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