G2 INTELLIGENCE

Diagnostic Testing & Technology Report

Competitive Intelligence & Analysis for an Expanding Global Market

Issue 3-13/March 2013

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Atomic Force Microscopy May Help Diagnose Cancer

Soft peaks identified in primary breast cancer tumor samples using an indentation-type atomic force microscope (IT-AFM) may serve not only as mechanobiological markers for the onset of cancer, but they may have prognostic qualities as well, according to a study published in the November issue of *Nature Nanotechnology*. These distinct stiffness profiles may provide evidence of more aggressive, potentially metastatic cells.

The researchers performed IT-AFM analyses in ex vivo breast tissues to correlate the cells' nanomechanical profiles to pathohistological findings in known normal, benign, and malignant biopsy specimens. Healthy specimens had a unimodal stiffness distribution of 1.13 kPa to 1.83 kPa. In benign lesions stiffness was uniform but values indicated a different stiffness composition (range from 1.91k Pa to 3.68 kPa). By comparison, a representative cancer biopsy typically exhibited a bimodal stiffness distribution with two prominent peaks at 0.57+0.16 kPa (peak 1) and 1.99+0.73 kPa (peak 2).

"Overall, nanomechanical profiling by IT-AFM provides quantitative indicators in the clinical diagnostics of breast cancer with translational significance," write the authors, led by Marija Plodinec from the University of Basel in Switzerland. For more information on how atomic force microscopy may be used in clinical laboratories of the future, please see *Inside the Diagnostics Industry* on page 5.

Initial MolPath Pricing Released by MACs Disheartening, True Impact on Dx Industry Uncertain, Experts Say

While laboratories are sure to be disappointed that the preliminary pricing unveiled for new molecular diagnostic Tier 1 Current Procedural Terminology (CPT) codes may be "slightly worse" than the 20 percent to 25 percent that the industry expected, it is too early to assess the extent of the impact on the diagnostics industry. While uncertainty never sits well, experts say reimbursement for molecular diagnostics will be "evolving" and the industry needs to strongly advocate for fair, value-based pricing.

In late January two Medicare administrative contractors (MACs)—Palmetto GBA (covering J1—California, Nevada, and Hawaii) and Cahaba (J10—covering Alabama, Georgia, and Tennessee)—announced pricing for many of the molecular diagnostic tests recently assigned CPT codes. Given the wide variability of prices and the variance in the number of procedural codes billed per similar tests under the "code stacking" reimbursement method used prior to January 2013, crosswalking prices of molecular tests was determined by the Centers for Medicare and Medicaid Services (CMS) to not be feasible.

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▲ **Initial MolPath Pricing Released by MACs Disheartening,** *from page 1*

Gap-filling procedures allow each MAC to determine local reimbursement rates for 2013, while for 2014 CMS will use the 2013 median carrier amount to set the national limitation amount. Palmetto's pricing schedule was expected to be closely examined. Given their extensive data collected in its Molecular Diagnostic Services (MolDx) program, Palmetto's pricing could serve as an example for other MACs.

However, the impact of Palmetto's reimbursement rates may be limited, says William Quirk, a senior research analyst for Piper Jaffray, in large part because Palmetto lost its MAC contract (Noridian will take over the region July 27). While Palmetto officials told a G2 audience they believe MolDx will be rolled out nationally, Quirk says the contract to retain control of the MolDx program is not finalized.

"The bottom-line is we think the new reimbursement rates create some noise for the clinical labs and diagnostic product companies, however the real impact is limited," writes Quirk in a Jan. 29 research note. "Given Palmetto's short tenure left running J1, which ends in July, we view Palmetto's reimbursement release as more hot air than a risk to industry pricing."

Early analysis of the pricing does demonstrate pricing reductions, frequently in excess of 25 percent. These cuts are likely to have some impact on laboratories' bottom line, with molecular diagnostic testing representing approximately 5 percent to 10 percent of total revenue for some laboratories and Medicare reimbursement for these codes estimated to account for 15 percent to 20 percent of that revenue.

Molecular Diagnostic Pricing Comparison ***(selected tests)***

Test	New CPT Code	Quest Stacked Price	Cahaba Price	Palmetto GBA Price (Coverage)	% Change (Palmetto/ Stacked Price)
BRAF gene	81210	$259.10	$123	$57.51 (Yes)	-78%
BRCA1, BRCA2 (breast cancer 1 and 2) gene analysis; full sequence analysis (hereditary breast and ovarian cancer)	81211		$2,900	No decision	
EGFR (epidermal growth receptor) gene analysis, common variants (non-small-cell lung)	81235	$301.92	$123	$116.25 (Yes)	-61%
KRAS	81275	$911.28	$235	$225.88 (Yes)	-75%
Warfarin Testing— 1. CYP2C9 gene common variants 2. VCorc1 gene	1. 81227 2. 81355	$205.94	1. $50 2. $90	1. $96.78 (Yes) 2. $83.19 (Yes)	-13%
CYP2C19 gene common variants (pharmacogenomic testing)	81225	$289.54	$305	$135.26 (Yes)	-53%

Source: Adapted from data provided by Piper Jaffray, XIFIN

Among the tests showing positive coverage decisions, but lower reimbursement rates than national labs previously received under code stacks, are genetic tests central to personalized medicine including variant testing for cancer-related genes BRAF, KRAS, and EGFR as well as several pharmacogenomics-related genes (see box). Several diagnostics manufacturers develop tests exposed to these cuts including Nanosphere, GenMark Diagnostics, and Qiagen, according to Quirk. Even still, he maintains positive or neutral financial performance expectations for these companies.

Qiagen's EGFR Companion Diagnostic

In mid-January Qiagen announced it submitted its therascreen EGFR test to the U.S. Food and Drug Administration (FDA) as a companion diagnostic to guide treatment for advanced non-small-cell lung cancer and identify which patients would be eligible for treatment with afatinib, a new investigational oncology compound developed by Boehringer Ingelheim, that targets the EGFR mutation.

The test and drug were developed in tandem. While the FDA has expressed interest in joint submissions, and it is presumed reimbursement for the test will be easier if jointly approved by the FDA, Qiagen's test is exposed to reimbursement cuts under the recently announced pricing.

Experts note another "concerning" trend in Palmetto's announcement. Darren Lehrich, a managing director at investment banking firm Deutsche Bank, says that so far the MAC is only covering testing for 53 of the 78 codes they have announced pricing for. Typically coverage is denied because of insufficient evidence of clinical utility. Among some of the denial of coverages by Palmetto are ApoE genotyping to assess cardiovascular risk, BluePrint Agendia's test to guide breast cancer therapy selection, Life Technologies' Pervenio Lung RS test to assess risk of early-stage lung cancer, and Biocept's OncoCee CTC Assay to detect metastatic disease for breast, prostate, lung, and colon cancer.

Coding consultants and industry advocates say it is imperative for laboratories and diagnostics developers to argue the case for both coverage enhancements and fee adjustments. Such requests for reconsideration will require evidence of clinical utility and direct costs for the test.

IVD Market to Pass $50 Billion in Global Sales in 2014

Sales in the global in vitro diagnostics (IVD) market are forecast to cross the $50 billion milestone for the first time in 2014, according to data compiled by the growth consulting firm Frost & Sullivan (Mountain View, Calif.). Test volumes will be driven upward by both an aging population battling more chronic and age-related diseases that require lab-based management as well as growth in key market segments including infectious disease testing and molecular diagnostics.

"The IVD market is dwarfed compared to other health care segments like the pharmaceutical industry. So $50 billion is monumental, in a sense, especially in light of cost cutting and testing commoditization," Jonathan Witonsky, principal life science analyst at Frost & Sullivan, tells *DTTR*. "Despite big constraints, it is noteworthy the IVD diagnostics market is continuing to grow and will surpass that nice, big, round number of $50 billion."

Macroeconomic forces and domestic changes in health care policy have been and will continue to affect this growth.

"We are anticipating in 2014 [the Patient Protection and Affordable Care Act], when fully implemented, will have a significant impact on the testing market," says Witonsky. "The rates that we are now anticipating over the next 24 months are in line with estimates made several years back, but the factors contributing to the growth aren't what was anticipated back then."

For instance, Witonsky says, before the height of the recession growth did not materialize as anticipated in certain testing sectors, like point-of-care (POC).

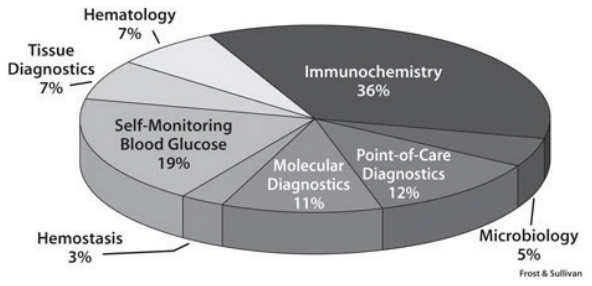
"For POC testing, prior to the downturn, we were expecting 8 percent to 10 percent growth. But in the height of the recession large populations of people lost benefits when they lost their jobs and didn't seek routine care the way they historically would have," Witonsky explains. "POC growth is at 5 percent to 6 percent, two-thirds of what was

previously anticipated. The growth rate is responding to a new model away from private practice to consolidated delivery and given the recession and macro-trends we aren't seeing the liberal spending on physicians' office laboratories we did a few years back."

Among Witonsky's forecasts for IVD market segments for the next two years are:

- **Tissue Diagnostics**—The highest rate of growth is expected in this $3 billion segment—12 percent compound annual growth rate (CAGR) between 2012 and 2014. Demand for automation, particularly in the preanalytical steps, will drive this growth. Witonsky cites that only 30 percent of the anatomic pathology laboratory is automated, compared to 80 percent of the clinical chemistry laboratory.

Global In Vitro Diagnostics Market (2012)



Source: Jonathan Witonsky, Frost & Sullivan

- **Molecular Diagnostics**—Molecular diagnostics, while still only comprising $5 billion of the IVD market, is expected to have a high rate of growth—11 percent CAGR. Witonsky expects a move away from screening panels toward genome sequencing.
- **Microbiology**—The $2.4 billion segment is forecast to grow at 4 percent CAGR. Emphasis will be on improving efficiency measured as time to results and gains in workflow productivity.
- **Hematology**—While slow 2 percent CAGR growth is expected in this $3 billion market segment, Witonsky says that manufacturers are likely to focus on improving analyzer accuracy, thereby reducing manual microscopic cell differentiation.

Ongoing Budget Debate Prolongs Tools' Uncertainty, Poll Finds

While Congress was able to temporarily avert the fiscal cliff, a Goldman Sachs survey conducted in January shows that research laboratories find little solace in the deal and remain cautious in the face of continuing budget uncertainty. Life science tool and consumables manufacturers must face the prospect that long-term laboratory spending remains highly dependent on political dealings and macroeconomic improvement, the researchers conclude.

A poll of 41 research labs in the United States (n=31), Germany (n=5), and the United Kingdom (n=5) found that year-over-year budget growth continued to decelerate in all regions during the fourth-quarter of 2012. U.S. laboratory budget growth declined from 11 percent in the second quarter to 5 percent in the fourth quarter.

"We believe this data demonstrates a downward sloping trend for U.S. budget growth and results can be attributed to a further deterioration in the overall funding environment," writes Isaac Ro, an analyst and vice president at Goldman Sachs in a research note detailing the survey results. "We note that significant uncertainty remains around whether or not postponement of sequestration will have any effect on 1QCY13 trends."

Respondents cited heightened importance toward the purchase of capital equipment in the latest survey with 68 percent of respondents indicating equipment purchases

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Imagining the Laboratory of the Future

What will the laboratory of the future look like? Will emerging technologies like atomic force microscopy find their way into diagnostics? Will a disruptive technology unimaginable today revolutionize laboratory processes, much the way few could have guessed smartphones' impact. Maybe more gradual advances will shape the future with incremental improvements in automation or perhaps a new delivery model will emerge for laboratory services.

DTTR spoke to a variety of laboratory experts to better understand potential trends that will shape the future of laboratories'. Experts all recognize that clinical laboratories future direction will be shaped by larger forces in health care delivery, information technology, and personalized medicine. The bottom line, they agree, is that the need for actionable clinical information is the underlying driver of clinical testing. How and where that testing will be performed is the big question.

"There will be continued centralization of sophisticated, high-value tests run on expensive platforms," says Robert Boorstein, M.D., Ph.D., founder of ClasGroup, a clinical laboratory consulting company. "It is not logical from an economic or clinical point of view for all labs to perform expensive, low-volume, complex tests. There will be more hub-and-spoke arrangements."

Boorstein points to the success of companies like Genomic Health and Myriad Genetics, which are able to meet the clinical needs of a "high-end market" from one centralized laboratory. He does not envision a future where every hospital laboratory will have sequencing analyzers given the investment needed in capital equipment, skilled technologists, and in bioinformatics expertise.

"It is not logical from an economic or clinical point of view for all labs to perform expensive, low-volume, complex tests."
—Robert Boorstein, M.D., Ph.D.

"Laboratories are information creators. As the information becomes more complex, so have to the lab solutions," says Marc Grodman, M.D., CEO, Bio-Reference Laboratories. "With the advent of genomic data we have access to more information with more clinical utility than we ever thought possible. . . . We need to take the strength of labs and develop software and clinical tools to prepare this more complex information."

While genomic interpretation will inevitably play a role in the future of medicine, there has been growing interest in laboratory tools that isolate and capture the desired rare cells necessary for genomic analysis. The ANGLE Group (United Kingdom) is nearing commercialization of its Parasortix cell capture technology. Parasortix is based on patented microfluidic separation techniques that the company says is broadly applicable, although its current focus is on circulating tumor cell (CTCs).

"To me the critical point where Parasortix fits into the laboratory of the future is that it extends the range of techniques for blood-based diagnosis," explains Shane Booth, Ph.D., a principal at ANGLE and the CEO of Parasortix. "In the current generation of blood-based diagnosis you identify biochemical markers. And that is OK for troponin or glucose, but not every disease provides a biochemical indicator. But by being able to identify very rare cells, sometimes present in concentrations as low as one in 1 billion, you have the technology catching up so that you can do molecular analysis."

Booth tells *DTTR* that to be incorporated into the future of routine pathology, technology must be easy to use, reliable, reproducible, efficacious, and efficient—"generally the faster the better," he says. Currently though, such efforts to automate are focused on biochemical analysis, more so than on cellular analysis. In alignment with this philosophy, Parasortix has spent the last year ensuring that the design of its first generation of commercial equipment is easy to use and fairly automated by integrating separate equipment into a single machine. The filtering cassette is consumable and has been designed to fit the standard dimensions of a lab microscope. Post-separation steps will be integrated into the machine, with semiautomatic immunostaining built in. The Parasortix product will be commercially available to the research market in early 2013 while the company works to secure clinical regulatory approval.

Another way to characterize cells may be rooted in a technology not currently widely applied to clinical diagnostics—atomic force microscopy (AFM)—which in just 10 years has evolved from an unconventional microscope into a multifunctional, nano-scale toolbox with potential clinical diagnostic applications, according to a review article published in *Nature Nanotechnology* by Daniel J. Müller, from Technische Universität Dresden (Germany). Originally invented to image the topography of surfaces, AFM is now recognized for its ability to characterize biological cells and their components with unprecedented resolution. Single biomolecules can be observed without the need for fixation or staining. Current AFM techniques allow a "lab-on-a-tip" with single-molecule analyses providing an understanding of various biochemical, physical, and chemical properties within the cell.

"The proposed nanosensors may become important medical diagnosis tools, leading to fast and reliable detection of biomarkers that reveal disease risk, progression or therapy response," writes Müller. "DNA, RNA, protein, or combinations thereof could be detected in parallel on a single cantilever array."

Interdisciplinary Approaches to Diagnostics Reshape Laboratories

New technology like AFM brings together researchers from multiple disciplines. While interdisciplinary approaches to tackling biomarker discovery and genomic understanding are not new, in recent years efforts to foster greater collaboration have begun to be tangibly witnessed in modifications to laboratory design.

In 2010 Novartis created its "labs of the future" program, which challenged researchers to approach their work "without regard to geographic, physical or scientific boundaries." Such a philosophy was manifested in the unveiling of the design changes at the Novartis Institutes for BioMedical Research in Basel, Switzerland, which became the company's prototype. Lab benches and workstations were designed to be modular, flexible, and reconfigurable to meet team needs. And a century-old tradition was broken, the company said, when chemists and biologists began working on projects side by side. Ian Baxendale, Ph.D., a professor at Durham University (United Kingdom), says this change in laboratory design circles back to the focus on personalized medicine.

"There is more intensive investment in small models targeting more specialized [niches] with integrated biological and chemical solutions. There is a closer working

relationship between biologists, chemists, and formulation people," Baxendale tells *DTTR*. "Because of this, laboratories cannot be designed in physical or geographic isolation. Focused teams are going to come together for a project. Projects are going to more quickly change direction and teams may be disbanded, rearranged, or set up for the next project. There is not the same economic model anymore to justify the price or the outlay for personalized medicine drugs."

Baxendale cites the trend in Europe to mobile trailers that are physically moved around and reconfigured to bring together the right teams for a particular project. Even in clinical laboratories there is evidence that more mobile approaches might reshape laboratory service delivery for routine testing.

A Shift in Delivery of Laboratory Services

"The truth is nobody really knows what technology or process may emerge that we hadn't even thought about yet," says Boorstein. "If we run back five to 10 years, there are things that are ubiquitous now, like the iPhone and the iPad, that we couldn't have predicted. Nobody in IT would have said that we would be using Apple products in a laboratory situation."

With enhanced communications and automation in the laboratory, Boorstein predicts there will be an inevitable movement in the industry toward standardization.

The advent of 'smart' laboratory tools could accelerate the push toward standardization.

"I was recently in a large commercial reference lab in Canada and if you look at the racks, every rack and every tube was the same. In Canada there is one tube type and size. In U.S. labs the processes accommodate a lot of unneeded diversity and variation that adds complexity and costs. How does that variability really help you?" Boorstein questions. "Every tube should fit in every slot and all instruments should be able to work with each other, just like every outlet takes all plugs."

The advent of "smart" laboratory tools could accelerate the push toward standardization. Researchers from Fraunhofer Institute for Biomedical Engineering (Germany) have developed smart test tubes in which a tiny microchip is embedded in the plastic of the test tube and used to store all relevant information, including the patient's name and when and where a sample is from. The microchip data can be edited. The smart test tube can carry the sample's entire history all the way through results with no need for a technician to write anything.

With the adoption of more uniform standards, Boorstein says, cookie-cutter laboratory delivery solutions may follow.

"Think cookie-cutter solutions the way Target or McDonalds work. There are clear standards and roles. Things are done the same way and done well and done multiple times. The lab industry would not be harmed by moving that way," says Boorstein. "Who would have thought that CVS and Wal-Mart could be major players in flu shots? Using cookie-cutter standards somebody could design a 500-square-foot to 1,000-square-foot laboratory that could do 90 percent of routine laboratory work. You could walk into a CVS with an order barcoded in your phone, just like you do at Starbucks, and you can have results in 30 minutes. Look to existing distribution channels—Wal-Mart, CVS, RiteAid, Walgreens—to optimize outpatient laboratory testing. It could be a game changer."

▲ **Ongoing Budget Debate Prolongs Uncertainty,** *from page 4*

were either their first or second most important priority, up from 54 percent in the previous quarter. Conversely, the number of respondents citing consumables as their purchasing priority decreased from 86 percent in the previous quarter to 73 percent in the most recent poll.

"We believe this is a function of both year-end spending and increased demand for new technologies during a delayed replacement cycle," writes Ro. "In general, we do not expect any significant rebound in spending until greater clarity is gained from the government on future budget cuts," but this pent-up demand could create "near-term upside for innovative equipment companies."

Ro continues that while "we continue to expect companies with large portions of revenue tied to consumables to maintain steadier streams of revenue in this uncertain funding environment, we believe any incremental funding that becomes available will go toward new equipment purchases."

Despite this lingering uncertainty, companies with high academic and government exposure including Illumina and Affymetrix preannounced fourth-quarter 2012 top-line results that were positive and in line, respectively, suggesting to Ro that improving macroeconomic conditions may have helped offset funding constraints. Looking forward, Ro and his colleagues say that "bellwether" players like Danaher and Agilent have given "tepid" 2013 organic growth guidance suggesting that the industry has "properly" set expectations ahead of earnings season, but he expects that "leaner cost structures and purchasing patterns are likely to be the new reality in academia."

Flu Epidemic Could Provide Boost to Diagnostics Industry

One good thing can come of the flu epidemic sweeping the United States—it could provide a positive earnings boost to diagnostics companies, experts say. Early fourth-quarter 2012 indicators show that the early outbreak drove up significant demand for Cepheid, Quidel, and Meridian Biosciences' products.

Based on historical data, analysts from Piper Jaffray's health care division say the outbreak will probably have a negligible impact on clinical laboratories but will likely "be a catalyst for positive earnings surprises over the next two quarters" for diagnostics companies, writes Kevin K. Ellich, a senior research analyst. According to U.S. Centers for Disease Control and Prevention data analyzed by the researchers from Piper Jaffray, between Sept. 30, 2012, and Jan. 5, 2013, roughly 80,000 specimens were tested and reported by collaborating laboratories. Compared to the same reporting period in 2010 (the last bad flu season), the current flu season already shows an 84 percent increase in the number of tests performed.

Among the companies already acknowledging the financial benefit to the outbreak:

- Quidel (San Diego) released preliminary fourth-quarter 2012 results that topped expectations with revenues between $53 million and $54 million.

  "We saw a sudden and early onset to this year's influenza season. I am pleased, however, with how well we were able to respond to the increased and rapid de-

mand for our flu tests," said Douglas Bryant, Quidel's chief executive officer, in a statement. "While QuickVue Influenza A+B sales benefited most from the patient visits for influenzalike illness in the quarter, Sofia Influenza A+B revenues were a contributor as well."

- Meridian Biosciences (Cincinnati) reported record first-quarter 2013 net sales of $45.4 million, an increase of 13 percent, from the same period of the prior fiscal year.

  "With the early start to the influenza season, not surprisingly, the respiratory category increased 42 percent, with flu accounting for $1 million of incremental revenues," said John Kraeutler, Meridian's chief executive officer, in a statement.

- Cepheid (Sunnyvale, Calif.), which has been plagued with "growing pains" related to manufacturing operations, was still able to grow clinical test revenue by 32 percent, including 25 percent growth in commercial business last year. The company ended 2012 optimistic about growth of its GeneXpert system and growing test menu.

  "All commercial products are now out of allocation with the exception of Xpert Flu which is seeing even higher demand than we anticipated," said Chief Executive Officer John Bishop on an earnings call. "Given the unexpectedly strong flu season and as we balance customer requirements and work to rebuild inventory here at the company, it is likely that Xpert Flu will remain on allocation during the first quarter. As a result, it is possible that a couple of our lowest-volume tests could experience intermittent windows of allocation as we prioritize Flu production." G2

Expanded Pap Test Can Detect Ovarian, Endometrial Cancers

Expanded analysis from a standard liquid-based Pap smear specimen can detect endometrial and ovarian cancers, according to a study published Jan. 9 in *Science Translational Medicine*.

The proof-of-concept findings are raising hope for the prospect of a broadly applicable genomic-based screening test capable of early detection of gynecologic malignancies. While the findings are not ready for clinical deployment, clinicians are encouraged that the "PapGene" sequencing-based test could parallel the success that Pap testing had on reducing rates of cervical cancer.

Through a combination of published mutations and newly generated findings from whole-exome sequencing, the researchers compiled a list of 12 genes representing common mutations in endometrial and ovarian cancers (APC, AKT1, BRAF, CTNNB1, EGFR, FBXW7, KRAS, NRAS, PIK3CA, PPP2R1A, PTEN, and TP53). Because of the small number of cancer cells in the Pap sample fluid compared to the DNA from normal cells in the endocervical canal, DNA templates were amplified with modified gene-specific primers.

The researchers used the PapGene multiplexed molecular assay and massively parallel sequencing to search for mutations in 24 endometrial and 22 ovarian known cancer cases. Mutations were detected in 100 percent of the endometrial cancers and in 41 percent (nine of 22) ovarian cancers. The authors say there was no obvious correlation between the fraction of mutant alleles and stage or histopathologic subtype of the cancer. No mutations were detected in the 14 healthy controls.

"The most important finding in this paper is that diagnostically useful amounts of cells or cell fragments from endometrial and ovarian cancers are present in the cervix and can be detected through molecular genetic approaches," write the authors, led by Isaac Kinde, a candidate for an M.D. and Ph.D. from Johns Hopkins University (Baltimore). "The test, even in its current format, appears to be promising as a screening tool for endometrial cancer because even the lowest stage endometrial cancers could be detected through the analysis of DNA in Pap specimens."

The authors say that potential improvements to the test, including increased number of gene targets or improved collection methods, could increase the sensitivity of the PapGene test and allow it to detect more ovarian cancers.

Many pieces are already in place for clinical adoption of such a screening test, the authors note. Screening is already incorporated into standard medical practice. DNA purification is identical to HPV evaluation that is already conducted, and amplification can be implemented on any massively parallel sequencing instrument. Analysis of the PapGene test is done "completely in silico," and the results are objective and quantitative. The researchers anticipate the cost of the test would be similar to current cervical fluid HPV testing.

Laboratories Identify New Tick-Borne Pathogen in the U.S.

Gene sequencing has led to the first documented cases of human infections with Borrelia miyamotoi, a tick-borne infectious agent related to Borrelia burgdorferi, the cause of Lyme disease. The first case report was published in the Jan. 17 issue of the *New England Journal of Medicine* (*NEJM*), but Imugen (Norwood, Mass.), a clinical laboratory specializing in vector-borne and blood-borne infections, says that several subsequent cases have been identified in the Northeast since the initial report.

B. miyamotoi was first identified in Japan in 1995. In the first documented case in the United States (an older, immunocompromised, febrile patient with progressive mental deterioration), the pathogen was identified using both microscopy and a polymerase-chain-reaction (PCR) assay.

Upon Giemsa and Gram staining distinct spirochetes bacteria were identified in cerebrospinal fluid (CSF), but not from a peripheral-blood smear. Antibody studies were conducted and immunofluorescence confirmed that the spirochete was a member of the genus borrelia. Genetic analysis was conducted with enhanced contamination-control practices and precautions. Extracted DNA from the patient's CSF was tested using a real-time PCR assay and genuswide borrelia primers.

Despite this being the first documented case, another report published in the same issue of *NEJM* by Yale researchers shows that serum samples from 1 percent to 3 percent of residents in the New England area where Lyme disease is endemic were reactive in an experimental serologic assay targeting the B. miyamotoi GlpQ antigen (an antigen that is nonreactive to B. burgdorferi antibody). This finding, the researchers say, suggests that exposure is actually relatively common and that B. miyamotoi may be prevalent in these areas.

"American demographic characteristics are changing, with a trend toward an increasingly older population, as well as extended survival of patients with human immunodeficiency

virus infection or cancer," writes case study lead author, Joseph L. Gugliotta, M.D., from the Robert Wood Johnson Medical School (New Brunswick, N.J.). "Immunocompromise in older patients should always prompt a more rigorous laboratory analysis, because such persons may serve as sentinels for poorly recognized or novel pathogens."

Salivary Gland Biopsy May Become First Tissue-Based Parkinson's Diagnostic

In what may be a breakthrough for tissue-based diagnosis of Parkinson's disease, two related studies, one in cadavers and a smaller one in living Parkinson's patients, show that biopsies of the salivary gland can consistently identify abnormal proteins associated with the disease.

"Diagnostic inaccuracy is a critical impediment to clinical trials and especially clinical trials at early-stage disease," write the authors from the Arizona Parkinson's Disease Consortium in an article published in the February issue of the *Journal of Neuropathology and Experimental Neurology*. "Even more importantly, for clinical trials and therapies that use invasive methods, such as deep brain stimulation, neural transplantation, or gene therapy (9Y17), misdiagnosis inevitably exposes considerable numbers of non-Parkinson's disease subjects to potentially damaging procedures without a therapeutic benefit."

Yet there is no diagnostic test for Parkinson's. Charles Adler, M.D., Ph.D., a study co-author, tells *DTTR* that current diagnosis hinges on clinical judgment and that diagnostic accuracy is roughly 80 percent, meaning that 20 percent of patients enrolled in clinical trials may not even have the disease. The researchers are "extremely encouraged" by the results of these proof-of-concept studies.

The researchers performed immunohistochemical staining for Lewy-type α-synucleinopathy (LTS) in samples from both large segments (simulating open biopsy) and needle cores of submandibular glands from 128 autopsied and neuropathologically classified subjects, including 28 with Parkinson's disease, 50 normal elderly patients, and the remainder with other diseases of the central nervous system. Immunoreactive nerve fibers positive for LTS were present in the large submandibular gland sections of all 28 subjects with Parkinson's and in 17 of 19 core samples (89 percent) in Parkinson's patients.

Following up on the these findings, the researchers then percutaneously performed core needle biopsies on the submandibular gland in living Parkinson's patients with advanced disease (greater than five years since diagnosis). The minor salivary glands were also removed for analysis. In findings released in advance of their presentation at the American Academy of Neurology annual meeting (San Diego; March 16-23) the needle biopsy was able to identify LTS in nine of 12 submandibular samples. Only one in 15 of minor salivary gland specimens was positive for LTS, making it a nonideal biopsy target.

Such immunostaining is not routinely done at laboratories at this point, Adler says, but similar techniques are used by academic neuropathologists on deep brain tissue specimens. The researchers will next conduct a similar study in early Parkinson's patients (less than five years from diagnosis) to test if it is possible to identify LTS in the submandibular glands earlier in the disease process.

G2 Insider

Selective D-Dimer Testing Strategy Improves Diagnostic Efficiency of DVT . . . A selective D-dimer testing strategy based on clinical pretest probability (C-PTP) for deep venous thrombosis (DVT) is safe and is more efficient than testing all suspected cases. The selective strategy substantially reduces the number of D-dimer assays performed on higher-risk patients and reduces the need for ultrasonographies in low-risk patients.

In a study published in the Jan. 15 issue of the *Annals of Internal Medicine*, consecutive symptomatic patients presenting for the first time with suspected DPT were randomized to either selective testing (n=860) or uniform testing (n=863). The selective testing strategy used D-dimer testing for patients with low or moderate C-PTP of DVT and used venous ultrasonograpy without D-dimer testing in patients with high C-PTP or inpatients. DVT was excluded at D-dimer levels less than 1 μg/mL in patients with low C-PTP or less than 0.5 μg/mL in those with moderate C-PTP.

The two diagnostic strategies resulted in a similar proportion of patients being diagnosed with venous thromboembolism (VTE) during initial testing and a similar incidence of symptomatic VTE at three months in both study groups. No patients with D-dimer levels between 0.5 μg/mL and 1 μg/mL (88 patients in the selective group and 81 patients in the uniform group) were diagnosed with VTE during initial testing or follow-up, confirming the safety of this approach, the authors say.

Selective testing reduced the proportion of patients receiving D-dimer testing by an absolute 21.8 percent. It also reduced the proportion who required ultrasonography by an absolute 7.6 percent overall and by an absolute 21 percent in outpatients with low C-PTP. Of the 360 outpatients with low C-PTP, results of D-dimer testing were negative in 288 patients and positive in 72 patients. Of the 72 D-dimer–positive patients, 11 percent were diagnosed with DVT by ultrasonography during initial testing and no others developed VTE during follow-up.

"Future studies to determine whether this diagnostic strategy can be applied to patients who present with suspected recurrent DVT or pulmonary embolism are required," write the authors, led by Lori-Ann Linkins, M.D., from McMaster University in Canada.

Company References

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March 2013

Diagnostic Testing & Technology Report (ISSN 1531-3786) is published by G2 Intelligence, 1 Phoenix Mill Lane, Fl. 3, Peterborough, NH 03458-1467. Tel: 800-401-5937. Fax: 603-924-4034. Web site: www.G2Intelligence.com.

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