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# DIAGNOSTIC TESTING & Emerging Technologies

**New Trends, Applications, and IVD Industry Analysis**

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## Exome Sequencing May Enable Prenatal Diagnosis, Management

Clinical whole-exome sequencing (WES) has been utilized for both pediatric and adult patients with complex, clinical presentations (like developmental disabilities) and typically yields a molecular diagnosis rate of about 30 percent. In these cases clinical WES is effective from both a cost and time perspective in ending long diagnostic odysseys. With shortening turnaround times, experts say that WES will soon be applicable in other patient populations, including critically ill neonates and even prenatally.

Two presentations at the American College of Medical Genetics and Genomics annual meeting (March 8-12; Tampa, Florida) demonstrate the potential for WES to diagnose genetic disorders prenatally, including aneuploidies, large chromosomal deletion and duplication syndromes, as well as single-gene disorders. Timely WES results can inform developmental prognosis, pregnancy management, and recurrence risk for subsequent reproductive planning.

*Continued on page 2*

## Utility of Post Op Lab Testing Questioned for Some Gynecologic Patients

Routine postoperative laboratory testing may be unnecessary in some gynecological patients, according to two abstracts presented at the Society of Gynecologic Oncology's (SGO's) Annual Meeting on Women's Cancer (March 19-22, San Diego).

The first study found that the likelihood of detecting postoperative laboratory abnormalities is low in asymptomatic patients following robotic hysterectomies in endometrial cancer patients. The researchers, led by Josephine Kim, M.D., an obstetrics fellow from the University of Chicago in Illinois, suggest that routine laboratory tests in asymptomatic patients following uncomplicated surgery may be unwarranted.

The researchers identified 380 patients without comorbidities who underwent uncomplicated surgery. The patients had a median age of 62 years, median body mass index of 32, and median length of stay of one day. All patients had at least one postoperative complete blood count.

*Continued on page 8*

## Exome Sequencing May Enable Prenatal Diagnosis, Management, *Continued from top of p.1*

Researchers from Baylor University (Houston) reported results from their first 43 consecutive prenatal cases. Fetal samples were obtained through either an invasive procedure or a product of conception.

In 34 cases, WES was performed for the proband only (proband WES) followed by Sanger sequencing studies of both parental samples. In nine cases, the trio all underwent WES. For the final report, Sanger sequencing was used to confirm all contributing changes for trio cases. WES was performed on the Illumina HiSeq2500 (average 11.4 Gb data per exome) with more than 97 percent of the targeted exome regions sequenced at a depth of 20X.

At Baylor the turnaround time was dramatically different for the two methods, with an average 12 weeks for proband WES (proband only WES followed by Sanger sequencing of parental samples) versus one to three weeks for prenatal trio WES.

The researchers found that the proband WES yielded a diagnosis of 32 percent (11 of 34) versus 33 percent in trio WES (three of nine). The turnaround time (TAT) was dramatically different for the two methods (average TAT for proband WES of 12 weeks versus one to three weeks for prenatal trio WES). When patients were classified by anomaly, diagnostic yields included:

- three of five cases of brain anomaly detected by ultrasound,
- six of 16 cases of brain anomaly and other organ system anomaly detected by ultrasound, and
- five of 22 cases of ultrasound anomaly not including the brain (e.g., cardiac, kidney, limb, and bladder anomalies, diaphragmatic hernia, heterotaxy, cystic hygroma, and edema).

Diagnosis was made for three of seven individuals with brain, cardiac defect, and other organ system anomaly. For 24 of the 43 cases this patient was the first presentation in the family and diagnosis was made for nine of these cases (38 percent). Of the 19 patients in which two or more family members had a similar phenotype, diagnosis was made for five cases (26 percent).

In a second presentation, researchers from GeneDx (Gaithersburg, Md.) shared WES results from 61 fetal samples. Twenty-six samples were from second trimester fetal terminations and demises and 16 samples were from third trimester demises.

Clinical indications for testing included fetal anomalies, fetal hydrops, intrauterine growth restriction, and discordant genetic and phenotypic fetal gender. The most common anomalies were seen in the central nervous system (22 cases), congenital heart disease (13 cases), cystic hygroma or hydrops (16 cases), facial anomalies (14 cases), and rocker bottom feet (nine cases). WES analysis was predominantly performed in fetus-parent trios (52 percent) or singleton cases (33 percent), with the remainder including fetus-parent duos or siblings.

The GeneDx researchers found that more than half of cases had prior reported normal karyotype (56 percent) or normal chromosomal microarray (69 percent).

Prior negative genetic testing was performed in five cases, including panel testing for Noonan syndrome, fetal akinesia deformation, lysosomal storage disease, and single gene testing for LAMB2, NPHS2, PLCE1, NPHS1, or FGFR3. Overall, 21 percent (n=13) of cases yielded a definitive molecular diagnosis, 34 percent (n=21) had reportable variants associated with a probable diagnosis, and 30 percent (n=18)

tested negative. Similar numbers of cases had one variant (n=20) and two or more variants (n=23) identified. FGFR2 was the only gene with pathogenic variants in more than one case (two fetuses with features of skeletal dysplasia).

Novel molecular diagnoses were made including MYH3-related distal arthrogryposis, PIK3CA-related megalencephaly-capillary malformation-polymicrogyria syndrome, L1CAM-related hydrocephalus, RIPK4-related Bartsocas-Papas syndrome, MRPS22-related mitochondrial dysfunction, PKHD1-related polycystic kidney disease, and CYP11A1-related adrenal insufficiency.

***Takeaway: Emerging research shows that WES performed prenatally yields similar diagnostic results as WES performed clinically in children and adults.***

## Genetic Predisposition to Stress Fractures ID'd in Healthy Individuals

Genetic predisposition may play a role in susceptibility to stress fractures, according to a study published in the March issue of *Purinergic Signalling*. Understanding the genetic predisposition may be important among military recruits and elite athletes who have an estimated incidence of stress fractures as high as 24 percent.

Genetic variants in the P2X7 receptor (P2X7R) gene, a key regulator of bone remodeling, have previously been tied to bone phenotypes and clinical outcomes in older populations. Variants have been tied to both gain and loss of function.

*"The precise mechanism by which these mutations may influence stress fracture risk is unknown but may include decreased sensitivity of bone to mechanical loading or decreased osteoclast apoptosis."*

—Ian Varley

The researchers studied P2X7R in two separate cohorts—210 Israeli Defense Forces (IDF) military personnel and 518 elite athletes from the United States and the United Kingdom. Among the IDF recruits, stress fracture injury was diagnosed in 43 personnel based on symptoms, evaluation by an orthopedic surgeon, and a positive bone scan.

Among the elite athletes medical imaging-verified stress fracture injuries were seen in 125 participants. Those in both cohorts not having stress fractures confirmed by bone scans were considered controls. Participants were genotyped for 12 functional single nucleotide polymorphisms (SNPs) within the P2X7R gene using a proprietary fluorescence-based competitive allele-specific polymerase chain reaction assay.

The researchers found that the variant allele of P2X7R SNP rs3751143 (Glu496Ala—loss of function) was associated with stress fracture injury, while the variant allele of rs1718119 (Ala348Thr—gain of function) was associated with a significantly lower occurrence of stress fracture injury in IDF recruits. These findings were validated in the elite athlete cohort.

"The precise mechanism by which these mutations may influence stress fracture risk is unknown but may include decreased sensitivity of bone to mechanical loading or decreased osteoclast apoptosis," write the authors led by Ian Varley, from Nottingham Trent University in the United Kingdom.

***Takeaway: While still needing validation in a larger population, among active, healthy people there may be a genetic explanation for predisposition to stress fractures. These findings could hold importance among military recruits and elite athletes prone to repeated mechanical loading.***

# Inside The Diagnostics Industry

## States Eye Expanding Roadside Drug Tests as Law Enforcement Implements Oral Fluid Testing Pilots

With the movement to legalize marijuana use for medical and/or recreational purposes gaining traction, states are calling for improved standards for marijuana testing, particularly as it relates to measuring drivers' drug-related impairment. But, the association between legal definitions of detection and the capabilities of tests to judge impairment is complex and not fully understood.

### New Legislation Under Consideration

**California**

All eyes are on California, the country's most populous state. California voters approved medical marijuana in 1996, and a measure to legalize recreational marijuana is likely to qualify for the Nov. 8 ballot. In anticipation of the possible legalization of recreational marijuana, legislators are working on regulations designed to crack down on drugged driving.

The California Senate Public Safety Committee will proceed with SB 1462, authored by Sen. Bob Huff (R-San Dimas), which would allow law enforcement officers to use roadside oral swab drug screening tests when there is probable cause that a driver is impaired because of suspicious or reckless driving and the driver has failed sobriety field tests. If the driver fails the test or refuses to take it, he or she would be taken to the police station for a blood test to measure the level and type of substances present in his or her system. Sen. Huff has said the legislation wouldn't mandate the use of oral swab tests, but it would set standards for law enforcement agencies and free up federal funding to help buy the equipment.

**Vermont**

In late April, the Vermont House passed H.228 allowing police to conduct roadside saliva-based test on drivers with suspected impairment. The bill, which is awaiting Senate action, also sets new limits above which a driver is considered intoxicated when the saliva test shows evidence of both alcohol and marijuana. The new blood-alcohol level would be 0.05, down from 0.08, when 1.5 ng of THC is also present. In addition to marijuana, the new saliva test would check for the presence of a half-dozen other drugs. Separately, lawmakers are exploring legislation legalizing possession of small amounts of marijuana.

The Governors Highway Safety Association (GHSA) released its *Drug-Impaired Driving: A Guide for What States Can Do* report last fall. It provided recommendations that states focus on efforts to improve testing, including testing all fatally injured drivers, standardizing testing protocols and procedures for roadside testing and laboratory testing, and validating roadside testing devices. These efforts, GHSA says, will need to be informed by further research on the effects of drugs on driving; the effectiveness of drugged driving per se laws; the accuracy, reliability and cost-effectiveness of drug detection tests; and the feasibility of establishing national standards for various controlled substances involved in drug-impaired driving.

"An accurate, reliable, and inexpensive oral fluid test device that could be used at the roadside would be very useful. It should be quick and easy to use and should detect the most common drugs that impair drivers," writes GHSA in its report. "If an oral fluid test were of evidential quality for some drugs it might reduce the need for blood tests. Research is needed to continue refining, evaluating, and eventually establishing standards for oral fluid test devices. Continuing research is [also] needed to determine if a useful marijuana breath test device can be developed."

### Laws Expanding Use, Testing

According to the National Conference of State Legislatures, medical marijuana is legal in 24 states in the United States, while recreational use of the drug is legal in Alaska, Colorado, Oregon,

# Inside The Diagnostics Industry

Washington, and Washington, D.C. An additional 16 other states have decriminalized possession of small amounts of marijuana, with legislation pending in additional state houses. This changing legal landscape, combined with recent studies assessing the prevalence of drivers with detectable drugs in their blood, indicates that an increasing number of drivers are using marijuana. But, local and state law enforcement departments don't have the necessary tools and standards to determine if drivers are impaired.

In response to growing drug use, states have implemented piecemeal regulation for impairment testing. GHSA says that:

- Nine states have zero tolerance for delta-9-tetrahydrocannabinol (THC), the psychoactive ingredient in cannabis or metabolites.
- Three states have zero tolerance for THC but no restriction on metabolites.
- Five states have specific per se limits for THC

Additionally, DUID.org, a coalition to combat drugged driving, says that 15 states have statutes permitting forensic oral fluid testing. While the political will is growing to tackle impaired driving, the technology of testing is lagging.

## Challenge to Testing

Alcohol spreads throughout the body from saliva and breath, eventually moving into the lungs and bloodstream. So a breathalyzer or blood test can say how much alcohol is present in the body.

Marijuana, however, works differently than alcohol. High THC blood levels do not necessarily correlate with the "highest" or most impaired point. Eating marijuana, as opposed to smoking it, also makes it less likely to show up in the blood. And since THC is fat soluble, it can quickly leave the bloodstream, while being stored in the body's fat. This stored THC can leak out, and a blood test might show elevated THC levels—even if a driver didn't smoke that day and is completely sober.

## Top Oral Fluid Devices Used in Roadside Pilots

The two most frequently piloted roadside oral fluid drug tests are made by Alere (Waltham, Mass.) and Dräger (Germany).

**Dräger DrugTest 5000**

The Dräger DrugTest 5000 was first commercially introduced in 2008 and consists of a sampling system and analyzer. The suspected impaired driver moves the top part of the collector briefly back and forth between his/her cheek and gums until the integrated indicator signals that enough of a sample has been collected, the company says. The officer then inserts the test cassette and cartridge directly into the analyzer. The Dräger DrugTest 5000 tests for commonly abused drugs including THC (the psychoactive chemical compound found in marijuana), amphetamine, methamphetamine, opiates, benzodiazepines, cocaine, and methadone. The company says the device is used currently by law enforcement in more than a dozen states.

**Alere DDS2**

The Alere DDS2 device consists of a test cartridge and 1.5 pound-electronic analyzer. The company says actionable screening results for six drugs (amphetamines, benzodiazepines, cannabis [THC detectable at 25ng/mL], cocaine, opiates, methamphetamine, and methadone) are available in five minutes. Minimal training is required due to the three-button user interface that provides on-screen feedback. Additionally, the analyzer can store 10,000 results, which can be printed at the end of each test or reprinted from the memory card. Test data also can be downloaded to the optional Alere Software Application Suite for enhanced data management capabilities. Barcode recognition enables identification of test panel, lot number, and expiration date of the test cartridge.

# Inside The Diagnostics Industry

Many long for the marijuana analogy to a blood-alcohol level, but evidence suggests this may be impossible. Compared to alcohol, defining and identifying impairment due to drugs is more complicated. In experimental settings, marijuana has been shown to impair psychomotor skills and cognitive functions associated with driving, including vigilance, time and distance perception, lane tracking, motor coordination, divided attention tasks, and reaction time. However, detection of the drug's presence in the body, its concentration, and its impairing effects are not well understood and can vary by person due to frequency of use and individual differences in metabolism.

*"An ideal test would be one using saliva or breath THC concentration that accurately signifies impairment in all impaired individuals and does not falsely capture unimpaired, chronic frequent cannabis users (i.e., people using medical marijuana legally) due to residual THC. Unfortunately, the feasibility of this has not been established."*

—Barth Wilsey M.D.

The federal government classifies marijuana as a Schedule 1 drug, meaning that it has no accepted medical use and possesses a high potential for abuse. This categorization impairs the ability of scientists to obtain federally sanctioned marijuana for research. But given the lack of research and the growing national use of the drug (both for medical purposes and recreationally) due to permissive state laws, the federal government is under pressure to reclassify marijuana and enable new research that will inform understanding of impairment and validate testing technologies.

"An ideal test would be one using saliva or breath THC concentration that accurately signifies impairment in all impaired individuals and does not falsely capture unimpaired, chronic frequent cannabis users (i.e., people using medical marijuana legally) due to residual THC. Unfortunately, the feasibility of this has not been established," Barth Wilsey M.D., from the University of California Center for Medicinal Cannabis Research, tells *DTET*. "We are thus working on methods that, in the aggregate, will inform officers of the likelihood of cannabis-related driving impairment (i.e., information garnered from fluid THC levels and performance-based testing)."

The Center for Medicinal Cannabis Research was recently awarded a $1.8 million project, commissioned by the California legislature, to improve methods for spotting drivers impaired by marijuana. Wilsey says the combination of oral or breath THC tests plus a performance-based field test will be the most expedient way to detect drug-impaired driving.

"In all probability, saliva will probably become the most prevalent bodily fluid for roadside screening," Wilsey's colleague Thomas Marcotte, Ph.D., adds. "There are currently commercial products that provide a readout (either positive or negative for defined levels of THC) within minutes. The rationale is that legislators and police officers will desire rapid analysis of driving under the influence of cannabis testing at the roadside, eliminating transport of detainees to hospitals or police stations for a blood draw to determine a blood level."

The researchers say that breath testing currently requires sending the absorbent material that collects the cannabinoids to the lab for analysis, limiting its utility as a roadside device.

# Inside The Diagnostics Industry

## Pilot Cases

Beginning in March 2015, Colorado State Patrol (CSP) troopers began piloting five oral fluid testing devices. The CSP declined to disclose the companies or specific devices being used while the pilot is ongoing, but preliminary results are expected soon. It was reported that the Colorado attorney general's office kicked in $233,747 to help purchase the devices statewide.

Participation in the pilot is voluntary and drivers have to consent. Currently, the swab is being used for research and only used after a suspect is arrested and blood is drawn, the current standard procedure for testing THC. The oral fluid testing devices are currently in CSP field offices.

While Colorado's roadside testing pilot is being closely watched nationally because of the state's legalization of recreational marijuana, pilot testing programs are occurring throughout the country, including in California.

*"Due to its broad reach, the DT5000 screening system has undergone extensive, independent, scientific validation and been utilized in approximately one million tests worldwide."*

—Michael Willis, president, Dräger Safety Diagnostics

In February, Dräger's oral fluid drug test results from its DrugTest 5000 (DT5000) mobile drug screening system were found to be scientifically reliable in a hearing for a vehicular manslaughter case in California. The judge's decision, which is the first scientific-reliability ruling for an oral roadside testing device in the United States, allowed for the defendant's DT5000 results to be presented to the jury. Legal experts say this is a landmark case for the use of admissible oral fluid drug test results in the court of law.

While an appeal is possible, if an appellate court upholds the decision regarding the scientific reliability of the oral testing device, the ruling on the admissibility of test results will be made binding throughout California.

"Due to its broad reach, the DT5000 screening system has undergone extensive, independent, scientific validation and been utilized in approximately one million tests worldwide," said Michael Willis, president of Dräger Safety Diagnostics, in a statement. "This first Kelly-Frye hearing, as well as the push by the Kern County District Attorney's Office for oral fluid result admissibility in the case, is confirmation that the DT5000 is on its way to becoming a standard screening method for DUID investigations and prosecutions."

The company says its DT5000 system is broadly used across Europe and Australia and is currently in field use or pilot programs by law enforcement agencies and Drug Recognition Expert (DRE) trained officers in more than a dozen states in the United States.

***Takeaway: Roadside testing for marijuana is needed at a time when driving under the influence is increasing. Yet, development of these tests and adoption is hampered by non-uniform testing standards and legal thresholds, as well as the current inability to differentiate the presence of a drug from impairment.***

## Utility of Post Op Lab Testing Questioned for Some Gynecologic Patients, *Continued from bottom of p.1*

While 54 percent of patients had abnormal postoperative hemoglobin values, only one patient (0.5 percent) required a blood transfusion for symptomatic anemia. Similarly, 54 percent of patients had abnormal postoperative white blood cell counts, but only two patients (1 percent) required intervention, both for symptomatic urinary tract infections. Results of complete blood counts alone prompted no intervention in asymptomatic patients.

> *"These results call into question the utility of performing routine hemoglobin testing after minimally invasive surgery for endometrial cancer."*
>
> —John Nakayama, M.D.

On the first postoperative day, the vast majority of patients (91 percent) underwent a basic metabolic panel. Potassium levels were corrected in 39 asymptomatic patients (11 percent) even though most (54 percent) fell in the normal range. Similarly, more than one-third of asymptomatic patients (37 percent) had corrective interventions for postoperative magnesium levels, despite nearly two-thirds (64 percent) falling in the normal range. Two asymptomatic patients had abnormal routine postoperative laboratory results that led to intervention—one for elevated creatinine consistent with acute kidney injury and one with hyponatremia.

The authors report that the charges for routine postoperative laboratory testing totaled $260,882 (an average of $782 per patient), suggesting that cutting these tests in asymptomatic patients could yield measurable savings.

In the second study, researchers from University Hospitals in Cleveland, Ohio, retrospectively identified 235 patients who underwent minimally invasive surgery for endometrial cancer (either standard laparoscopy [LSC] n=138 or robotic-assisted surgery n=97) from 2010 to 2015. Baseline demographics and perioperative characteristics were evaluated. Clinical hemodynamic instability was defined as development of tachycardia, hypotension, low urine output, or dizziness.

The researchers found that patients with a lower body mass index were significantly more likely to have a greater decrease in hemoglobin, while surgical history, surgery/anesthesia time, blood loss, lymphadenectomy, use of preoperative anticoagulation, and use of postoperative ketorolac were not associated with greater decreases in hemoglobin. In total, 52 patients (22.1 percent) had at least one sign or symptom of hemodynamic instability postoperatively. Clinically symptomatic patients were significantly older and had significantly greater decreases in hemoglobin, compared to asymptomatic patients. Only symptomatic patients (n=5) required postoperative blood transfusions.

"These results call into question the utility of performing routine hemoglobin testing after minimally invasive surgery for endometrial cancer," writes co-author John Nakayama, M.D. "Hemoglobin testing may only be necessary for patients who develop signs or symptoms of hemodynamic instability. Omission of this routine test in asymptomatic patients could result in sizable health care cost savings. In addition, these results provide more data that could be used to support the safety of same-day discharge after minimally invasive surgery for endometrial cancer in appropriately selected patients."

***Takeaway: New evidence shows that postoperative blood tests may be unnecessary following some gynecologic surgeries. Reducing this unnecessary testing could yield cost savings, and possibly shorter discharges for some patients.*** G2

# Prospective Trial Shows Utility of Liquid Biopsy for Lung Cancer

Current clinical practice calls for assaying all patients with nonsquamous lung cancer for activating mutations in the EGFR gene and for ALK fusions to identify potential responders to targeted inhibitor therapies. Eventually, though, patients treated with these inhibitors will likely develop treatment resistance. While second-line treatments are available, the molecular mechanism of resistance must be identified and repeat biopsies are necessary.

There is much heralding of the potential for liquid biopsies that use tumor-derived cell-free DNA to revolutionize tumor genotyping, particularly for lung cancer where rapid, noninvasive means could improve care.

"The absence of reliable prospective data on the use of specific plasma genotyping assays in advanced non–small-cell lung cancer has left key aspects of its utility largely undefined and slowed its uptake as a tool for clinical care in patients with both newly diagnosed NSCLC and EGFR acquired resistance," writes Adrian Sacher, M.D., in an April 7 study published in *JAMA Oncology*.

*"Even with a diagnostic sensitivity of less than 100 percent, such a rapid assay with 100 percent positive predictive value carries the potential for immense clinical utility."*

—Sacher and Dana-Farber Cancer Institute

The results from this study, the first prospective study of plasma droplet digital polymerase chain reaction (ddPCR), shows that liquid biopsy technology can detect EGFR and KRAS mutations rapidly with high specificity. The authors say the assay is ready to be used for clinical decision making in selecting therapy and avoiding repeat biopsies.

Participants (62 percent female; 84 percent white) were either newly diagnosed planning for initial therapy (n=180) or developed acquired resistance to an EGFR kinase inhibitor and were planning for rebiopsy (n=60). Participants underwent initial blood sampling and immediate plasma ddPCR for EGFR and KRAS hotspot mutations between July 2014 and June 2015. All patients underwent biopsy for tissue genotyping, which was used as the reference standard for comparison. Rebiopsy was required for patients with acquired resistance to EGFR kinase inhibitors and in 22 patients with newly diagnosed NSCLC to obtain sufficient tissue to complete genotyping, highlighting one of the problems with tissue-based genotyping in lung cancer.

The researchers found that median turnaround time (TAT) for plasma ddPCR was 3 days (range one to seven days), while tissue genotyping took a median TAT of 12 days for patients with newly diagnosed NSCLC and 27 days for patients with acquired resistance. Plasma ddPCR demonstrated a positive predictive value of 100 percent for EGFR 19 del, 100 percent for L858R, and 100 percent for KRAS, but a lower 79 percent value for T790M. The sensitivity of plasma ddPCR was 82 percent for EGFR 19 del, 74 percent for L858R, and 77 percent for T790M, but lower (64 percent) for KRAS. Sensitivity for EGFR or KRAS was higher in patients with multiple metastatic sites (specifically for those with hepatic or bone metastases). No false-positive results were seen for driver mutations in EGFR or KRAS.

"Even with a diagnostic sensitivity of less than 100 percent, such a rapid assay with 100 percent positive predictive value carries the potential for immense clinical utility," writes Sacher and Dana-Farber Cancer Institute (Boston) colleagues. "A key limitation of plasma ddPCR is that although this method is adept at rapidly detecting specific targetable mutations, it cannot easily detect copy number alterations

*"There are many laboratories and companies working to develop assays that will assess presence or level of ctDNA in plasma, and most have concentrated on disease-specific panels."*

—P. Mickey Williams, Ph.D.

and rearrangements. … This limitation may potentially be addressed by using targeted next-generation sequencing of cfDNA for broad, multiplexed detection of complex genomic alterations including ALK and ROS1 rearrangements."

The lower limit of detection is 5 to 50 mutant copies in a background of 10,000 wild-type copies, and the assay has a dynamic range of 4 orders of magnitude.

"There are many laboratories and companies working to develop assays that will assess presence or level of ctDNA in plasma, and most have concentrated on disease-specific panels," writes co-author by P. Mickey Williams, Ph.D., from the Frederick National Laboratory for Cancer Research in Maryland in an accompanying editorial. "At this time, there are no standards or agreed-on approaches for controls and calibrators for assays of ctDNA. Furthermore, there are no agreed-on methods for reporting ctDNA burden (e.g., copies per milliliter of blood versus total copies, or a ratio to wild-type sequence) that would allow results from different assays and laboratories to be compared. The clinical utility of mutation detection in ctDNA relies on accurate quantitation, so these issues must be resolved."

***Takeaway: This is the first prospective study to show that a ddPCR-based liquid biopsy assay can rapidly and accurately detect EGFR and KRAS mutations in a real-world clinical setting so as to direct clinical care.***

## Cotesting Best for Cervical Cancer Screening, But Cytology Doesn't ID Other Gynecological Cancers

Despite the fact that the Pap smear has been around for 70-plus years and is credited with greatly reducing the incidence of cervical cancer, screening remains imperfectly executed in the United States. Adherence to guidelines is suboptimal and plagued nationally by a combination of overscreening, underscreening, and poor management of women with abnormal test results. Lack of guideline adherence is in part driven by confusion regarding the use of conventional cytology testing versus newer cotesting strategies (Pap cytology plus human papillomavirus [HPV] DNA testing).

Two abstracts presented at the Society of Gynecologic Oncology's (SGO's) Annual Meeting on Women's Cancer (March 19-22; San Diego) address economic and clinical benefit considerations of cervical cytology screening.

In a population partially vaccinated against HPV, a cotesting strategy has the highest screening costs, but also the lowest cervical cancer incidence and mortality, according to an abstract presented at SGO by Catherine Popadiuk, M.D., from of Memorial University of Newfoundland in Canada.

Popadiuk and colleagues used the Cancer Risk Management Model-Human Papillomavirus (CRMM-HPV) Canadian population microsimulation model to assess three screening strategies in a vaccinated population (HPV types 6/11/16/18). The three strategies included: triennial Pap smear in 25 to 69 year olds (PAP3); triennial Pap smear in 25 to 29 year olds with HPV DNA testing every five years from ages 30 to 69 years (PAP3/HPV5), and triennial Pap smear in 25 to 29 year olds and Pap/

HPV cotesting every 5 years from ages 30 to 69 years (cotest). The model assumed that the vaccination rate was 70 percent for girls aged 12 years when the program started in 2007 and that there was a 70 percent participation rate for screening in eligible women beginning in 2015.

The researchers found that cotesting cut cervical cancer incidence and mortality per 100,000 by 5.8 percent on average, compared with the Pap smear between 2015 and 2050. Over a lifetime, PAP3/HPV5 and cotesting resulted in 2.3 percent and 7.5 percent fewer cases than PAP3, respectively, plus 2.9 percent and 7.9 percent fewer deaths compared with Pap3, respectively. Cotesting was projected to require the most annual colposcopies (n=201,800 versus 148,900 and 102,600 for Pap3 and Pap3/HPV5 strategies, respectively). When considering lifetime costs of vaccination, cervical screening, and treatment, cotesting was the most costly ($27.98 billion), while PAP3/HPV5 was the least costly ($20.48 billion). Considering just direct screening costs, cotesting remained the most expensive strategy ($23.22 billion).

*"Failure to diagnose otherwise unsuspected endometrial or ovarian cancer has been described as one potential reason to avoid replacing cotesting with primary HPV screening."*

—Alexandra Freeman, M.D.,

While the benefits of screening to cut the incidence of cervical cancer are undeniable, regardless of screening strategy, researchers presented findings suggesting that cervical cancer screening is unwarranted for detecting endometrial or ovarian cancers, according to a featured poster presented by Alexandra Freeman, M.D., from Kaiser Permanente San Francisco Medical Center, San Francisco.

The Kaiser Permanente researchers identified 1,545,126 women who underwent cervical cytology screening in the health care system from 2009 through 2014. Abnormal cervical cytology was defined as: atypical glandular cells, other (normal postmenopausal endometrial cells), or malignant. Endometrial and ovarian cancer cases were confirmed using a local cancer registry over the study period. Laboratory databases, including reasons for the visit based on the history provided with the Pap requisition, were reviewed to determine any symptoms of endometrial or ovarian cancer.

The researchers found that over the five years 0.3 percent of women had abnormal glandular cell cytology results. Over the same period there were 3,898 primary invasive endometrial cancers and 1,434 primary invasive ovarian cancers. Among women diagnosed with endometrial and ovarian cancer, 5.0 percent and 0.9 percent, respectively, had abnormal cervical cytology in the 12 months prior to their cancer diagnosis. Visit notes indicate that 58.7 percent and 61.5 percent of those with abnormal cytology were symptomatic for endometrial and ovarian cancers, respectively at the time of screening.

"Failure to diagnose otherwise unsuspected endometrial or ovarian cancer has been described as one potential reason to avoid replacing cotesting with primary HPV screening," the authors write. "Performance of cervical cytology on 1.5 million women for the purpose of detecting the number of occult endometrial or ovarian cancers described above does not seem warranted."

***Takeaway: Cotesting (cervical cytology screening plus HPV DNA testing) remains costly, but is the most effective strategy for cutting the number of cases and deaths from cervical cancer. However, questions remain about the utility of cytology for diagnosing other gynecological cancers.***

G2 INSIDER

# Low Histopathologic Agreement for DCIS, Atypia Breast Cancer

Pathologists disagree with one another's interpretations about 8 percent of the time when diagnosing a single breast biopsy slide, according to a study published March 22 in the *Annals of Internal Medicine*. The present study applied previous discrepancy findings from the Breast Pathology Study (B-Path) to patient populations. Discordance between individual pathologists and a reference consensus diagnosis was more likely in cases of ductal carcinoma in situ (DCIS) or atypia, with higher levels of overinterpretation of disease risk. The authors say that this "diagnostic grey zone" needs to be considered in clinical management decisions. Histopathological diagnosis remains the gold standard of breast cancer diagnosis, despite concerns about the variability in specimen interpretations in clinical practice. B-Path previously found that one in four breast biopsy results were discordant with expert reference consensus diagnosis. But, experts note the study included higher proportions of cases of DCIS and atypia than typically seen in clinical practice. However, the study did not assess population impact.

The present study estimated the effect of interpretation variation from the perspective of U.S. woman having a biopsy. The researchers calculated predictive values using Bayes' theorem, combining results from B-Path with published data of the population-based prevalence of breast pathology diagnoses in women aged 50 to 59 years. B-Path compared 115 pathologists' interpretations of a single biopsy slide (6,900 total interpretations from 240 distinct cases) versus a reference consensus interpretation from three experts.

The researchers found that the reference panel members' review showed concordance with the final consensus diagnoses 90 percent of the time versus participants' 75 percent concordance. Overall, using one representative slide per case, 92.3 percent of breast biopsy diagnoses would be verified by reference consensus diagnoses, with 4.6 percent of discordant results overinterpreted and 3.2 percent underinterpreted.

Most U.S. women undergoing breast biopsy in clinical practice receive a benign without atypia diagnosis. For these women, diagnostic agreement with the reference panel would be high (97.1 percent). Verification of invasive breast cancer was also highly probable (97.7 percent). The likelihood that a diagnosis of atypia or DCIS would be verified by the reference consensus diagnosis was low. Diagnostic agreement with the reference consensus panel for atypia was less than 50 percent regardless of the pathologists' desire for a second opinion or whether they noted that the case was borderline. Using a single slide for the cases interpreted as DCIS, the reference consensus panel would interpret 9.5 percent as benign without atypia, 9.0 percent as atypia, and 11.8 percent as invasive breast cancer.

"Women with borderline breast lesions that are difficult to categorize, such as atypical ductal hyperplasia and low-grade DCIS, may benefit from revised guidelines for clinical treatment and management given the degree of diagnostic variability and biological overlap between these diagnostic categories," write the authors led by Joann Elmore, M.D., from University of Washington in Seattle.

## *Company References*

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