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New Trends, Applications, and IVD Industry Analysis

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IN THIS ISSUE

Test Quality: New Study Casts Doubt on Whole Exome Sequencing's Accuracy in Diagnosing Genetic Disorders

Depending on the reporting laboratory, patients who undergo whole exome sequencing may receive false negatives or incomplete test results. That is the finding of a new study published in *Clinical Chemistry* on Jan. 6, 2020. Conducted by researchers from the University of Texas Southwestern Medical Center, the study reviewed clinical tests from three major U.S. laboratories and concluded that whole exome sequencing routinely fails to adequately analyze large segments of DNA, a potentially critical deficiency that could prevent doctors from accurately diagnosing potential genetic disorders ranging from epilepsy to cancer.

Challenges Posed by Whole Exome Sequencing

Whole exome sequencing is a technique for analyzing protein-

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Genetic Testing: ACMG the Latest to Come Out Against Genetic Testing for All Breast Cancer Patients

The debate over the need for genetic testing of all breast cancer patients for germline pathogenic or likely pathogenic variants in BRCA1, BRCA2 and other breast cancer-linked genes continues with the American College of Medical Genetics and Genomics (ACMG) declining to recommend general testing. However, the ACMG did recommend evaluating all breast cancer patients for *their need for* germline genetic testing to assess their inherited risk for the disease.

Lack of Consensus on Need for Genetic Testing of Breast Cancer Patients

Of all cancers that impact women in the U.S., breast cancer has the highest incidence, regardless of race or ethnicity with an estimated

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producing genes. It is used to identify genetic mutations that cause disease in children and adults with rare or undiagnosed diseases. Of course, when parents and children undergo whole exome sequencing, they fully expect the test results to be accurate. So do the physicians who order the tests.

But exome sequencing may not be living up to those expectations. The process of fully analyzing the approximately 18,000 genes in an exome is inherently difficult and susceptible to oversights. This could explain why approximately half the tests do not pinpoint a mutation.

The Study

The researchers said they conducted the study because to test their hypothesis that vast differences in testing quality are endemic in clinical genetic sequencing but have not been well documented or shared with clinicians. The research team double checked findings from 36 patients' clinical exome tests performed from 2012 to 2016 by three major, unidentified U.S. clinical laboratories, noting coverage of genes and nucleotide positions. The study sample included 20 proband patients and 16 parents seen at a pediatric genetic testing clinic.

Although the sample size was admittedly small, the researchers said that the laboratories are representative of clinical genomics laboratories in the U.S. "I consider the three labs in the study to be of generally high quality, and we routinely send patient samples to these labs," researcher **Dr. Jason Park**, PhD, an associate professor of pathology at UT Southwestern, said in an email to *LabPulse.com*.

Insufficient Gene Coverage

The study results raise concerns about the accuracy of whole exome sequencing. First, the reanalysis showed that, on average, each laboratory adequately examined less than three-quarters of the genes—34%, 66% and 69%. It also revealed startlingly wide gaps in the laboratories' ability to detect specific disorders.

The researchers also found starkly contrasting results and inconsistency in terms of which genes were completely analyzed. A gene was not considered completely analyzed unless the laboratory met an industry-accepted threshold for adequate analysis of all DNA that encodes protein, which is defined as sequencing that segment at least 20 times per test. Notably, less than 1.5 percent of the genes were completely analyzed in all 36 samples. A review of one laboratory's tests showed that 28 percent of the genes were never adequately examined and only 5 percent were always covered. Another laboratory consistently covered only 27 percent of the genes.

One possible explanation for this wide variance in coverage was that each of the three laboratories used different methods and products for whole exome sequencing. Technology and/or reagent issues can result

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in variation between laboratories, including the method used to turn a patient's DNA into a library of fragments and the probes used to capture the DNA of interest, Park explained. But, he added, variation in quality of results within a laboratory is related to laboratory practices, and individual laboratories can and should address the variation between samples being tested.

Whole Exome Sequencing: Comparison of Test Results from 3 Major U.S. Labs

	Technology	Coverage
Laboratory A	VCRome v2.0 (Roche Sequencing Solutions) or xGen Exome Research Panel v1.0 (Integrated DNA Technologies)	69% (12,184 of 17,723 genes)
Laboratory B	VCRome v2.1 (Roche)	66% (11,687 of 17,723 genes)
Laboratory C	SureSelect XT2 All Exon v4 (Agilent) or Clinical Research Exome (Agilent)	34% (5,989 of 17,723 genes)

Recommendations for Improvement

The authors called on laboratories to continue working to improve the consistency of exome-testing performance. Recommendations include:

Statistical measures: The use of average measurements in large datasets such as exome/genome is only meaningful when the average is further described in combination with other summary statistics such as standard deviation and coefficient of variation," according to the authors. "Additional statistics such as genes completely covered will also allow a more accurate assessment of the test's validity and would pressure clinical laboratories to achieve higher consistency."

Auditing and Inspection: The researchers also recommended increased auditing of coverage quality and overall performance by accreditation and regulatory inspectors. One example would be to incorporate evaluation of coverage consistency between laboratories into the College of American Pathologists (CAP) proficiency testing program for next-generation sequencing.

Takeaway

New evidence suggesting that whole exome sequencing routinely fails to adequately analyze large segments of DNA raises new doubt about whether physicians can rely on the method to accurately diagnosing potential genetic disorders. The problem is exacerbated by wide variation in accuracy from laboratory to laboratory.

Unless and until coverage and consistency improves, ordering physicians need to be aware of the deficiencies in whole exome sequencing. The

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New Study Casts Doubt on Whole Exome Sequencing's Accuracy in Diagnosing Genetic Disorders, *from page 3*

researchers recommended that physicians ask laboratories about which genes are covered in the tests and consider the alternative of targeted panel tests for some patients, e.g., for a child with epilepsy without other complicating clinical problems. For such patients, ordering a smaller genetic test that completely analyzes a panel of genes associated with that disease may not only be less expensive, but just as likely to help physicians find answers.

FDA WATCH

Agency Exempts Dozens of Tests from 510(k) Review

On Dec. 30, 2019, the U.S. Food and Drug Administration (FDA) published a final order exempting dozens of Class I and Class II medical devices that previously required 510(k) submission from 510(k) premarket review. **Practical Impact:** Effective Jan. 1, 2020, the newly exempted medical devices and test systems will no longer have to comply with the premarket clearance as long as certain conditions are met.

FDA 510(k) Medical Device Clearance

Under current FDA rules, new diagnostic tests are regulated like medical devices and thus need a premarket submission before they can be brought to market. Submission for low risk devices is generally via the 510(k) pathway which requires demonstrating that the device is at least as safe and effective as—aka, substantially equivalent to—an already legally marketed device that is not subject to premarket approval. Under the 2016 Cures Act, the FDA must publish lists identifying devices that no longer require clearance. The FDA had published such lists in 2017. The final order makes those lists final and incorporates them into the regulations.

Exempted Devices

More precisely, the FDA identified a list of class I devices and class II devices now exempt from premarket notification requirements, subject to certain limitations. The list includes over 200 class I and II devices ranging from clinical chemistry and toxicology tests to cardiovascular, dental, hearing, neurological, orthopaedic and radiology products. Among the exclusions are 37 clinical chemistry and clinical toxicology devices including:

- Acid phosphatase (total or prostatic) test system;
- Angiotensin converting enzyme (A.C.E.) test system;
- Aspartate amino transferase (AST/SGOT) test system;
- Glucose test system;
- Continuous glucose monitor secondary display;

- Iron (non-heme) test system;
- Lactate dehydrogenase isoenzymes test system;
- Methylmalonic acid (nonquantitative) test system;
- Total thyroxine test system;
- Uric acid test system; and
- Breath-alcohol test system.

Also listed as being exempt are drug detection systems used for employment, insurance or federal drug testing programs, but only if they are used only for those purposes. Examples:

- Amphetamine test systems;
- Barbiturate test systems, benzodiazepine test systems;
- Codeine test systems; and
- Methamphetamine test systems.

The final order cautions that an exemption from the 510(k) process "does not mean that the device is exempt from any other statutory or regulatory requirements unless such exemption is explicitly provided by order or regulation."

Takeaway

Medical device and diagnostic testing firms need to review the new exemptions lists to determine if any of their current or proposed products are now exempt, or if products for which a 510(k) application is pending are now exempt and within any exemption limitation.

New FDA Approvals

Key new product approvals announced from late December 2019 through mid-January 2020:

Manufacturer(s)	Product(s)
Geneoscopy	Breakthrough device designation for colorectal cancer screening test based on extraction of stool-derived eukaryotic RNA transcripts
KDx Diagnostics	Breakthrough device designation for URO17 Bladder Cancer Recurrence Test
Luminex	Clearance for NxTag Respiratory Pathogen Panel qualitative test run on firm's Magpix instrument to simultaneously detect and identify nucleic acids from multiple respiratory viruses and bacteria
Microgenics	Clearance for Cedia Benzodiazepine Assay, a homogeneous enzyme immunoassay to detect benzodiazepines in human urine at cutoff concentration of 200 ng/mL

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Manufacturer(s)	Product(s)
Sentinel Diagnostics	Clearance for Albumin BCP assay measuring albumin in human serum or plasma
i-Sens	Clearance for ReliOn Premier Classic Blood Glucose Monitoring System measuring glucose in fresh capillary whole-blood samples drawn from fingertips, forearm, palm, thigh or calf
Promisemed Hangzhou Meditech	Clearance for Promisemed Heel Blood Lancet for collection of capillary blood from heels of newborn or premature babies
Immunotech Vit Oburka	Clearance for Active Free Testosterone RIA test radioimmunoassay used to measure free testosterone in human serum
Applied BioCode	510(k) clearance for BioCode Respiratory Pathogen Panel (RPP) for use on BioCode MDx-3000 system
Qiagen	Clearance for Therascreen PIK3CA RGQ PCR Kit as companion diagnostic to identify advanced breast cancer patients with PIK3CA mutations likely to respond to Novartis' Piqray (alpelisib)
Applied BioCode	510(k) clearance for BioCode respiratory pathogen panel diagnostic test used with firm's MDx-3000 system for detecting common respiratory viruses and bacteria
AstraZeneca + Merck	Expanded indication for olaparib (Lynparza) as a maintenance treatment for metastatic pancreatic cancer patients identified via a companion diagnostic
Curetis	Clearance for Unyvero LRT lower respiratory tract application cartridge
DiaSorin Molecular	Clearance for Simplexa VZV Swab Direct assay to detect varicella-zoster virus (VZV) DNA from cutaneous + mucocutaneous swab specimens
PerkinElmer	De novo premarket clearance for GSP Neonatal Creatine Kinase-MM kit for use in newborn screening for Duchenne Muscular Dystrophy

Industry Guidance: AACC Calls on Laboratories to Take Measures to Minimize Risks of Biotin Interference

Biotin interference with test accuracy has become a matter of growing concern in recent months. Last November, the U.S. Food and Drug Administration (FDA) updated a previous safety communication warning laboratory personnel, diagnostic test developers, providers and patients that biotin can lead to incorrect laboratory test results. And now the laboratory community has responded with the American Association for Clinical Chemistry (AACC) issuing new guidance to help clinicians, laboratory professionals and patients keep biotin from interfering with test results.

Biotin Blinding

Biotin, or Vitamin B7, is a water-soluble vitamin commonly used as an ingredient in multi-vitamins, prenatal vitamins and dietary supplements that are marketed for hair, skin and nail growth. Biotin's property of bonding with specific proteins that can be measured to detect certain health conditions, make it a natural for laboratory testing. For example, biotin is a key ingredient in many immunoassays.

The problem with biotin-based detection methods and technology is that biotin can also distort laboratory test results and generate both false highs and false lows. This is especially true when testing is performed on patients that consume high levels of biotin. And because of B7's widespread use in commercial products, high biotin consumption is far from unusual. At least 20 percent of Americans take biotin in some form, according to the AACC.

FDA Reaction to Biotin Interference

In 2017, the FDA issued a safety communication addressing biotin interference with certain *in vitro* diagnostic tests and has since issued recommendations for laboratory personnel and test manufacturers to minimize the potential for interference. The FDA expressed specific concern about biotin interference resulting in falsely low levels of troponin—the biomarker that aids in diagnosis of heart attacks. Misleading diagnoses as a result of incorrect laboratory results could lead to potentially serious clinical implications, the FDA cautioned.

Since the 2017 safety communication, some laboratory test developers have been successful at minimizing biotin interference of their assays, according to the updated safety communication, but others have not yet addressed it. The troponin problem remains of particular concern as the FDA continues to receive adverse events reports indicating that biotin interference caused falsely low troponin results.

Accordingly, the agency resounded the alarm in November by issuing new and more specific guidance. For more details, see *Diagnostic Tests & Emerging Technologies (DTET)*, Jan. 2, 2020.

AACC Biotin Communication Recommendations

The AACC *Guidance Document on Biotin Interference Laboratory Tests* guidance explains the mechanics and extent of the problem in clinical detail. It calls on laboratories to determine which of their immunoassays may be affected by biotin interference and educate clinicians and patients about their findings. Recommended methods of communicating with the former include the use of formal memos and laboratory bulletins and participation in conferences and clinical rounds. Methods of notifying

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AACC Calls on Laboratories to Take Measures to Minimize Risks of Biotin Interference, *from page 7*

patients could include the use of placards in outpatient phlebotomy centers, with information on biotin use. Other suggestions:

- Healthcare systems could include questions about the use of biotin and nutritional supplements during the inpatient/outpatient registration process;
- Clinicians could ask patients about their use of food supplements and explain the relevance;
- Electronic health records could be updated to display an alert to clinicians at the time of test order whenever tests with known biotin interference are ordered; and
- Clinicians should contact the laboratory when laboratory results do not fit the patient's clinical picture or if the patient is known to have consumed a dose of biotin of more than 5 mg.

AACC Verification Recommendations

The AACC says laboratories should also take measures to verify suspected biotin interference. Recommended methods:

- Use of a specimen diluent for the assay;
- Removal of excess biotin via streptavidin-coated beads; and
- Biotin quantification via chromatography (LC-MS/MS) or other procedure.

Ideally, a specimen suspected of biotin interference should be analyzed with a different assay that does not use biotin in its format, according to the AACC guidance. Alternatively, the laboratory may request a new specimen after the patient has abstained from biotin for a time.

Recommendations for Patients

Patients also need to play a role in preventing biotin interference, starting with being aware of the problem and their own biotin intake levels. The AACC's recommendations:

- Patients who have consumed 5–10 mg biotin should wait at least eight hours before having blood collected for laboratory tests;
- Longer washout periods of up to 72 hours may be required to prevent interference in assays with interference thresholds <30 ng/mL (<122.8 nmol/L); and
- Unless medically contraindicated, patients prescribed a high-dose biotin therapy (≥100 mg/day) should abstain from biotin for at least 72 hours before blood collection. ;

Laboratories and clinicians also need to keep in mind that patients with renal impairment may exhibit higher circulating biotin concentrations and prolonged elimination rates. The AACC also recommends against

reporting patient results after dilution or biotin removal unless the procedure has been validated by the laboratory.

Takeaway

"The recent increase in the use of high-dose biotin supplements requires that laboratorians and clinicians be mindful of the potential for biotin interference in biotinylated immunoassay-based laboratory tests," noted the AACC guidance document authors **Danni Li**, **Angela Ferguson**, **Mark Cervinski**, **Kara Lynch** and **Patrick Kyle** in a statement. "Ideally, manufacturers will reformulate assays that are sensitive to biotin. Given that the timeline from initial assay design to reformulation and governmental approval requires months to years, laboratorians and clinicians will have to remain mindful of this issue for some time to come … [and] should work together to ensure accurate laboratory results."

UK Study: Targeted Genetic Risk-Based Prostate Cancer Screening Would Save the Most Lives at the Best Costs

Targeted screening of men at high genetic risk of the disease could prevent nearly one in six prostate cancer deaths. That is the tantalizing finding of a new computer modeling study led by the University College London (UCL).

The Prostate Cancer Diagnostic Challenge

Prostate cancer is the most common form of cancer in men, claiming more than 10,000 lives per year. But screening for prostate cancer is more problematic than screening for its sister diseases, breast and cervical cancer. The effectiveness of current prostate-specific antigen blood testing is marred by the PSA protein's lack of reliability as a biomarker. Thus, while high PSA levels denote prostate cancer, the cancer is often low grade and poses no threat to the patient. High PSA may also indicate infection, inflammation or other disease. But because of the risks involved, physicians commonly order biopsies to rule out prostate cancer for patients whose screening tests show high PSA levels. Ultimately, a large percentage of these biopsies prove unnecessary.

The Study

These diagnostic problems explain why the UK has a national screening program for cervical and breast cancer but not for prostate cancer. With this in mind, UCL researchers performed a study computer modeling the harms and benefits of introducing four-yearly PSA screening for two groups:

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Targeted Genetic Risk-Based Prostate Cancer Screening Would Save the Most Lives at the Best Costs, *from page 9*

- All men ages 55 to 69; versus
- A more targeted checks for those at higher genetic risk of the disease, nearly half of the men in the above age group.

The study, published in *PLOS Medicine*, created a hypothetical cohort of performing different kinds of screening on 4.5 million men, the number of men aged 55 to 69 in England. Outcomes including prostate cancer deaths averted, unnecessary diagnoses and screening costs were compared against:

- No screening;
- Universal age-based screening; and
- More targeted screening using a range of genetic risk thresholds.

The simulated scenarios posited that men ages 55 to 69 would have four-yearly checks once they reached the risk threshold. This would mean a widening proportion of men having checks the older they got, as older men are at greater risk of the disease.

The Study Results

The researchers found that testing the population at higher risk of prostate cancer yielded the biggest health benefit in terms of both preventing prostate cancer deaths and minimizing unnecessary treatments for harmless tumors.

The optimal scenario, the study found, would be to screen men with a 4% to 7% risk of getting prostate cancer over the next 10 years, i.e., between roughly half and a quarter of all men ages 55 to 69.

Screening *all* men in that age group would result in the most deaths averted (20%); but it would also lead to extra cost and a large number of unnecessary diagnoses, with nearly one in three cancers detected by screening proving harmless.

Screening at a threshold of 4%, by contrast, would prevent 15% of (nearly one in six) deaths from prostate cancer while delivering the greatest gains in terms of quality adjusted life years, meaning more years of good health across the population. As compared with screening all men ages 55 to 69, screening at the 4% threshold would also reduce the number of unnecessary diagnoses of harmless cancers by about one third.

Screening men with a 4% to 7% risk would also be much more cost effective than screening all men ages 55 to 69, saving between one fifth (for the 4% risk threshold) to nearly half of the cost (7% risk threshold), while maintaining the benefits of screening.

Takeaway

"Prostate cancer is a leading cause of death from cancer in men in the UK, but screening is not performed because the harm of overdiagnosis is thought to outweigh the benefits," noted senior study author Professor **Nora Pashayan** (UCL Applied Health Research).

"Our study shows that targeted screening can reduce unnecessary diagnoses while helping to prevent people dying from the disease by enabling earlier detection."

It should also be noted that prostate cancer screening in the UK has historically been utilized less frequently than in the US.

ACMG the Latest to Come Out Against Genetic Testing for All Breast Cancer Patients, *from page 1*

271,270 new cases and 42,260 deaths in 2019. Despite the longstanding availability of testing for inherited cancer, including the BRCA1/2 genes for which testing has been clinically available for more than two decades, only a small proportion of the at-risk population has traditionally been tested.

The ACMG statement, which the nationally recognized genetic medicine professional organization published in its official journal, *Genetics in Medicine*, on Dec. 13 is contrary to a consensus guideline issued by the American Society of Breast Surgeons in February 2019 which does recommend genetic testing for BRCA1/2 and PALB2, and where clinical factors and family history dictate, for other genes.

However, the ACMG statement is consistent with the recently updated National Comprehensive Cancer Network (NCCN) guidelines recommending the use of patients' own cancer history and family history to determine the appropriateness of genetic testing assessing the risk of breast, ovarian and pancreatic cancers. For the details, see *Diagnostic Tests & Emerging Technologies (DTET)* Jan. 2, 2020.

There is insufficient evidence to recommend universal genetic testing for BRCA1/2 alone or in combination with multi-gene panels for all breast cancer patients, notes the lead author of the ACMG statement, **Tuya Pal** of Vanderbilt University Medical Center.

What the ACMG Did Recommend

While not coming out for universal testing, the ACMG made a point to emphasize that it "considers germline genetic information to be critical to the management of patients with genetic conditions." Accordingly, it did recommend that all women with breast cancer be evaluated for the need for genetic testing based on existing clinical criteria, including:

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ACMG the Latest to Come Out Against Genetic Testing for All Breast Cancer Patients, *from page 11*

- Age at diagnosis;
- Family cancer history;
- Expression of estrogen progesterone receptors; and
- HER2 expression.

The ACMG noted that such testing is not being provided, citing estimates that less than 10 percent of adults with BRCA1/2 pathogenic or likely pathogenic variants in the US have been identified, and less than 20 percent of breast and ovarian cancer patients who should receive testing according to guidelines are actually receiving it.

Furthermore, the ACMG recommends that clinicians:

- In discussions with patients, be aware of the current insufficient evidence to support genetic testing for all patients with breast cancer;
- After identifying a pathogenic or likely pathogenic mutation in moderately penetrant breast cancer genes, recognize that guidance is based on consensus recommendations and that enhanced screening, to date, has not been associated with enhanced survival or earlier stage diagnosis;
- Whenever genetic testing is performed on a clinical basis, ensure it includes full gene sequencing and is conducted in a CLIA-certified or CAP-accredited laboratory.

Takeaway

Skepticism clearly remains on the clinical utility and cost-effectiveness of genetic testing for all breast cancer patients. The ACMG urged professional societies to work together to advance harmonized, evidence-based recommendations and reduce barriers to care. "Testing alone will not improve outcomes but rather implementation of appropriate care following testing is required," the organization wrote.

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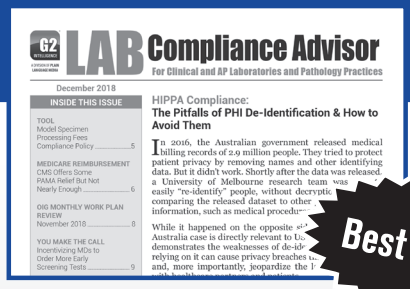
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