G2 INTELLIGENCE
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DIAGNOSTIC TESTING & Emerging Technologies

New Trends, Applications, and IVD Industry Analysis

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Top of the News: Rapid, Genomic-Based Surveillance IDs Aids Hospital Infection Control

Routine genomic sequencing can identify and inform hospital infection control personnel of patient transmission events in near real-time, enhancing not only detection, but follow-up and investigation for better outbreak control, according to a study published April 23 in *Infection Control & Hospital Epidemiology*. The integration of genomic and clinical epidemiologic data analyses detected transmission clusters not identified with standard surveillance of nosocomial infections.

Currently, investigation of suspected health care-associated infectious transmissions requires manual surveillance of case

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Top of the News: Technical Factors behind Discordant Results with Liquid Biopsies

Technical factors are a major source of variation in liquid biopsy test results, according to a study published online March 14 in the *Journal of Clinical Oncology*.

While biological factors, including low rates of tumor shedding and tumor heterogeneity are known to complicate analysis, the authors highlight that technical factors may be an underappreciated source of testing errors and raise "clinical concern" for false positive (FP) and false negative (FN) tests results.

"Although our study demonstrated that the majority of tumor-plasma discordance is a result of technical factors, with continuous improvement over time, NGS should approach the state of genotyping technology in which the majority of discordance is attributable to biologic factors such as tumor heterogeneity and clonal hematopoiesis of indeterminate potential," write the authors led by **Daniel Stetson**, from AstraZeneca in Boston, Mass.

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Rapid, Genomic-Based Surveillance IDs Aids Hospital Infection Control, *from page 1*

clusters by infection control personnel, followed by strain typing of clinical and environmental isolates in suspected clusters.

To rapidly detect transmission clusters, the researchers assessed the effectiveness of infection control surveillance using whole-genome sequencing (WGS) of microbial pathogens to identify potential transmission events for epidemiologic review. Prospective sampling of clinical isolates at a single academic medical center occurred from Sept. 1, 2016, to Sept. 30, 2017. Surveillance cultures for methicillin-resistant S. aureus (MRSA) and vancomycinresistant enterococci were routinely obtained on admission and weekly in the seven adult intensive care units (ICUs), the pediatric ICU, the neonatal ICU, and the bone marrow transplant unit. This study included one isolate per body site per patient per day.

Strains of Staphylococcus aureus, Enterococcus faecium, Pseudomonas aeruginosa, and Klebsiella pneumonia were evaluated. Isolate genomes were sequenced. Single-nucleotide variants were analyzed and a cloud-computing platform was used for WGS analysis and cluster identification through genomic analysis with linkage to geospatial and temporal data from integrated medical records. The clinical infection control department staff conducted a retrospective manual chart review to determine whether the clinical evidence supported the transmission of the genomically related bacterial isolates between patients.

The researchers report that 1,257 isolates with a positive culture for the species of interest were received from 1,073 patients. Sequencing was successful for 823 patients (87 percent inpatients). Most strains of the four pathogens were unrelated. However, 34 potential transmission clusters (n = 96 patients) were identified.

Chart review found that nine clusters had obvious clinical associations that were identified retrospectively. Only one of these clusters was suspected with routine, manual surveillance. Cross-transmission occurred in both the inpatient and outpatient settings. The authors note that the characteristics of the potential clusters were complex and likely not identifiable by traditional surveillance alone.

While the average cluster had 2.9 patients, the largest genetic cluster, included 21 MRSA isolates from 13 patients who all had community-onset MRSA infections. Six of these patients shared a history of recent or current intravenous (IV) drug use, one patient was an emergency medical technician who may have had occupational contact with IV drug users, and two patients with no prior history of IV drug use were followed for chronic medical conditions by the same clinical service. Four additional patients had no history of IV drug use and no obvious clinical connection to the other cases.

"The integration of clinical data is essential to prioritize suspect clusters

DTET

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for investigation, and for existing infections, a timely review of both the clinical and WGS results can hold promise to reduce health care-associated infections," write the authors led by **Doyle Ward,** Ph.D., from University of Massachusetts, Worcester. "A richer understanding of cross-transmission events within health care settings will require the expansion of current surveillance approaches."

The cloud-computing approach has potential to inform infection control practice "proactively," the authors say, noting that WGS analysis of a cultivated isolate can be performed in less than 48 hours and their cloud computing platform can analyze and generate potential relatedness matches in about 3 hours.

Takeaway: Integration of genomic and clinical epidemiologic data can augment infection control with nearly real-time surveillance for to identify of infectious transmission events within the health care setting. Rapid expanded surveillance may ultimately improve patient safety and lead to health care savings.

Emerging Tests: Spectroscopy IDs Potential Metabolic Markers to Diagnose Fibromyalgia

The identification of metabolic patterns in the blood of patients with fibromyalgia may improve diagnosis and enable discovery of targeted treatments, according to a study published Feb. 15 in the *Journal of Biological Chemistry*.

Fibromyalgia is part of a larger group of chronic pain syndromes (e.g., chronic fatigue syndrome and irritable bowel syndrome), but definitive diagnosis remains a challenge due to the lack of reliable biomarkers. Currently, doctors rely on patient-reported symptoms and a physical evaluation of a patient's pain, focusing on specific tender points.

"Unfortunately, no reliable diagnostic test for fibromyalgia exists," write the authors led by **Kevin V. Hackshaw**, M.D., from Ohio State University in Columbus. Such a test would be a significant step towards earlier diagnosis of and intervention for this condition, helping to improve patient outcomes, contain health care and/or legal costs, and potentially provide clues to the etiopathogenesis of the syndrome."

The researchers used dried blood spots of peripheral blood samples (derived from fingersticks) from patients with a diagnosis of fibromyalgia (n = 50), rheumatoid arthritis (n = 29), osteoarthritis and (n = 19), and systemic lupus erythematosus (n = 23). Bloodspot samples were analyzed using vibrational microspectroscopy (a portable FT-IR and FT-Raman microspectroscopy) and also underwent metabolomics analysis by ultra high-performance liquid chromatography (uHPLC), coupled to a photodiode array (PDA) and

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Spectroscopy IDs Potential Metabolic Markers to Diagnose Fibromyalgia, *from page 3*

tandem mass spectrometry (MS/MS).

Vibrational spectroscopy measures the energy level of molecules enabling rapid, high-throughput, and non-destructive analysis. Analysis produces a characteristic chemical 'fingerprint' with a unique signature profile with spectra separated into discrete clusters that permit classification of individuals based on subtle physiological differences, the authors say. The patterns are based on the "predictable ways" different functional groups absorb infrared light.

The researchers report that FT-IR and Raman spectroscopies produced distinct clustering of the specimen samples according to their disease class with no misclassifications. This discrimination power, the authors say, was dominated by vibrations of the backbone in proteins and nucleic acids, in addition to mineral differences in blood. Additionally, the spectra correlated (for both IR and Raman) with fibromyalgia pain severity measured with a validated questionnaire.

From each disease group, 10 randomly selected samples were analyzed by uHPLC- PDA-MS/MS. This technology also was able to distinguish between disease groups with certain metabolites existing in significantly different proportions as witnessed on the ultraviolet-visible light chromatograms.

"We found clear, reproducible metabolic patterns in the blood of dozens of patients with fibromyalgia," said Hackshaw. "Our studies have great importance both from development of a reproducible biomarker as well as identifying potential new therapeutic targets for treatment."

The authors say the next phase of their research will include an expanded study of 150 to 200 patients per disease group to see if the findings can be validated in a larger, more diverse population. The hope is to have a test ready for widespread use within five years.

Takeaway: Spectroscopy technology has identified metabolic patterns from dried blood spots that may lead to an objective diagnostic test for fibromyalgia.

Testing Trends: HCV Testing Uptsake "Limited" Even Among Baby Boomers

Only one in six baby boomers report having been tested for hepatitis C virus (HCV), despite recommendations for universal screening, according to a brief report published in the April issue of *Clinical Infectious Diseases*. Further, sociodemographic disparities further hamper HCV testing both among baby boomers and younger adults.

HCV is a public health concern, given both increasing incidence of infection among young people injecting drugs as part of the opioid crisis and increasing HCV-related morbidity and mortality among persons born between 1945 and 1965, who have had "clinically silent" infections and have remained undiagnosed for years.

Previous studies have shown that in order to reach national goals for reducing HCV incidence and HCV-related mortality, an estimated 70,000 to 110,000 new cases will need to be diagnosed each year until 2030.

In the present study, researchers used data from the National Health Interview Survey (2013 to 2017) to identify adults born between 1945 and 1994. Participants were asked, "Have you ever had a blood test for hepatitis C?" Responses were analyzed for participants answering either yes or no (n = 120 539).marginally

The researchers found that HCV testing coverage increased between 2013 and 2017 both among respondents born between 1966 and 1994 (13.2 percent to 16.8 percent) and respondents born between 1945 and 1965 (12.3 percent to 17.3 percent). Despite statistically significant increases, the authors classified these changes as only marginal. There were no increases in HCV testing over time among respondents without health insurance in any age group. HCV testing coverage was lower than for either hepatitis B virus and HIV testing among all age groups.

Among baby boomers, HCV testing coverage was significantly lower among females, individuals with less than a high school education, and foreign-born persons. Testing coverage was highest in the West. Further, those with military health insurance and public or government health insurance had higher HCV testing coverage than baby boomers with private health insurance.

"In addition to interventions to improve HCV screening in traditional health care settings, integrating HCV testing programs into nontraditional settings (e.g., nursing homes, emergency departments, and methadone clinics) and implementing community-based programs may be key strategies to expand coverage of HCV testing," write the authors led by **Eshan Patel**, from Johns Hopkins University in Baltimore, Md.

Takeaway: Rates of HCV testing remain low even among baby boomers, a cohort for whom universal testing is recommended. Rates are even lower for some sociodemographic subsets of age-based cohorts.

Testing Trends: Rule-In Using High-Sensitivity Cardiac Troponin May Misdiagnose Heart Attacks

One in 20 patients without clinical suspicion of acute myocardial infarction has a high sensitivity cardiac troponin I (hs-cTnI) level greater than the recommended upper limit of normal (ULN), according to a study published March 13 in *BMJ*. The authors say that the assumption that a result greater than the recommended threshold is a heart attack is flawed and can lead to inappropriate care. The findings show that the true 99th percentile for a general hospital population is not only inconsistent with the recommended ULN, but can vary by patient age and sex.

"The notion of using a single binary value greater than the supplied ULN of any assay to diagnose whether a patient has had an acute myocardial infarction is flawed," write the authors led by **Mark Mariathas**, from the University Hospital Southampton NHS Foundation Trust in the United Kingdom. "It is important for frontline clinical staff to understand that using a single cutoff of hs-cTnI to diagnose acute myocardial infarction might be inappropriate and that the ULN of the assay depends on the setting and the clinical characteristics of patients."

A joint definition released by the Joint European Society of Cardiology, American College of Cardiology, American Heart Association, World Heart Federation Task Force defined acute myocardial infarction as the rise or fall in cardiac troponin concentration with at least one value greater than the 99th centile derived from a reference population of healthy individuals. Further compounding interpretation of hs-cTnI results, the adoption of highly sensitive assays into clinical practice enables detection of troponin at much lower levels than previously possible.

"Using the 99th [per]centile to help rule out a diagnosis of acute myocardial infarction is clear cut and is based on a 'healthy' reference population," writes Mariathas and colleagues. "However, the recommended threshold and its application to patients presenting to hospital to rule in acute myocardial infarction is problematic, particularly when the degree of suspicion is low and other factors might contribute to the cardiac troponin concentration."

The present study measured Hs-cTnI concentrations among 20,000 consecutive inpatients and outpatients undergoing blood tests for any clinical reason from June 29, 2017, through Aug. 24, 2017. The Beckman Coulter Access AccuTnI+3 assay (Brea, CA, USA) was used and the company funded the study.

The researchers found that the 99th percentile of hs-cTnI for the whole cohort was 296 ng/L versus the manufacturer's ULN of 40 ng/L. Hs-cTnI concentrations were greater than 40 ng/L for 5.4 percent of all patients tested. When excluding 122 participants diagnosed with acute myocardial infarction and the 1,707 for whom hs-cTnI was requested for clinical reasons, the 99th centile was 189 ng/L for the remaining 18,171 patients.

The 99th percentile varied by clinical setting: 563 ng/L for inpatients and

65 ng/L for outpatients. More specifically, patients from the emergency department had a 99th percentile of 215 ng/L, with 6.07 percent greater than the recommended ULN. Among patients in critical care units, 39.0 percent and 14.16 percent of all medical inpatients had an hs-cTnI concentration greater than the recommended ULN.

Significant independent predictors of a patient having an hs-cTnI concentration greater than the 40 ng/L recommended ULN included advancing age, male sex, and decreasing estimated glomerular filtration rate. Additionally, almost twice the proportion of patients in their 60s had hs-cTnI concentrations greater than the ULN versus patients in their 50s. Levels also tended to be higher in men than in women.

"These results have important clinical implications that are almost certainly relevant to the application of all modern hs-cTn assays," the authors conclude. "Using the recommended ULN as a 'rule in' test for acute myocardial infarction might not be appropriate in patients presenting with atypical symptoms and other comorbidities, such as in the emergency department or on acute medical and surgical wards. This approach could expose patients to inappropriate pharmacological and invasive treatments that have only been shown to be beneficial in true type 1 myocardial infarction populations."

Takeaway: Use of the recommended ULN to diagnose acute myocardial infarction may be flawed. The true 99th percentile for a hospital population may be inconsistent with the recommended ULN, and can further vary by patient demographics.

INSIDE THE DIAGNOSTICS INDUSTRY

Partnerships, IT Solutions Improve Infectious Disease Diagnostic Stewardship

Appropriate use of testing is becoming a greater challenge, given the growing number of available diagnostic tests and their increasing complexity. While unnecessary or inappropriate testing can lead to wasted resources, unneeded tests can impact patient outcomes too, with potentially downstream repercussions including invasive medical workups or unneeded courses of antibiotics.

There is currently particular focus on the inappropriate use of testing tied to diagnosis and management of infectious diseases in hospitalized patients. Given concerns over antibiotic resistance, so called diagnostic stewardship—the process of ordering, performing, and reporting diagnostic tests to improve the treatment of infections and other conditions—is an area where laboratories, infectious disease clinicians, pharmacists, and leaders of antibiotic stewardship programs are working together to find solutions to improve test utilization management and interpretation of test results.

Diagnostic stewardship represents a convergence of interventions—preanalytic through

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postanalytic—that often rely heavily on information technology (IT) capabilities, such as clinical decision support, electronic ordering, and integration of laboratory results into electronic health records (EHRs).

DTET examined a recent sampling of studies demonstrating the impact of IT on laboratory test management.

EHR Alerts Can Change Physicians' Ordering

Programming a hospital's EHR to both inform providers on appropriate use of a costly gastrointestinal panel and to block unnecessary orders can substantially cut inappropriate testing, according to a study published April 23 in *Infection Control & Hospital Epidemiology*. Use of a simple hard stop alert in the EHR also was associated with significant cost savings.

Researchers hardwired University of Nebraska Medical Center's EHR to provide informational best practice alerts, as well as a hard stop that prevented inappropriate orders for the FilmArray Gastrointestinal Panel (GIPP; BioFire; Salt Lake City, Utah). Alternative stand-alone testing is offered, if necessary, but an override of the GIPP hard stop requires approval of the microbiology laboratory director. Test order patterns were compared among patients hospitalized with diarrhea for whom GIPP testing was ordered pre- and post- implementation of the EHR intervention.

The GIPP test panel is useful for new patients who may have been exposed to a wider variety of pathogens, and holds the potential to improve antibiotic stewardship and cut back on serial testing, as well as labor-intensive stool culture methods. However, it may be overused in the inpatient setting, where the authors say, pathogens other than C. difficile and norovirus are uncommon. Inappropriate GIPP ordering was defined as more than one GIPP per admission or in patients hospitalized for more than 72 hours.

The researchers found that in the pre-intervention period, 1,587 GIPP tests were ordered over 212,212 patient days (a rate of 7.48 per 1,000 patient days) versus 1,165 GIPP tests over 222,343 patient days during the post-intervention period (5.24 per 1,000 patient days), yielding a 30 percent reduction in total GIPP ordering rates between the 2 periods. Additionally, the rate of inappropriate tests ordered decreased significantly from 21.5 percent to 4.9 percent. Over the 15 months, the estimated cost savings reached up to $168,000.

"Consistent with published literature, we found that stool testing in patients hospitalized for more than 72 hours or those who have previously been tested is unlikely to be clinically relevant, which suggests that restricting repeat GIPP testing in the inpatient setting is reasonable," write the authors led by **Jasmine R. Marcelin**, M.D. "Our results highlight the value of diagnostic stewardship in antimicrobial stewardship programs and collaboration with the microbiology laboratory."

Order Entry Format Impacts Urine Culture Testing

Computerized physician order entry (CPOE) format can play an important role in reducing

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the burden of unnecessary urine cultures, according to a study published in the March issue *of Infection Control & Hospital Epidemiology*.

Urinalysis and urine culture tests are often ordered for patients without clinical suspicion of urinary tract infection, leading to unnecessary testing, increased hospital costs, and unnecessary antibiotic treatment. Previous research shows that treatment of asymptomatic bacteriuria does not affect patient outcomes.

Researchers from Barnes-Jewish Hospital in St. Louis evaluated the impact of changes to urine testing orderables in the hospital's CPOE system on urine culturing practices. A staged intervention to clarified test names and made changes to the urine reflex test panel. Urine culture rates were compared pre- and post-intervention among hospitalized adults who had at least one urine culture performed during their stay.

The researchers found that over the study period, 18,954 inpatients had 24,569 urine cultures ordered (11,780 during the pre-intervention versus 7,174 during the post-intervention period)— a 45.1 percent unadjusted decrease in the rate of inpatient urine cultures performed. In the post-intervention period, the urine culturing rate decreased significantly overall and for all specimen types, but the reduction in the urine culture rate was most marked for the catheterized (75.6 percent) versus clean-catch specimens (37.8 percent).

Overall, just over one-fourth of urine cultures (27 percent) were positive. There was a 16.4 percent increase in the proportion of positive urine cultures and a 6.9 percent decrease in the proportion of isolated urine cultures obtained.

"Our intervention resulted in an estimated cost savings of ~$104,000 for inpatient laboratory costs after implementation. This represents a fraction of the total costs and does not reflect the costs saved based on the medical decisions (e.g., delayed hospital discharge) and antimicrobial therapy," write the authors led by **Satish Munigala**, from Washington University in St. Louis, Mo. "In an era of reducing reimbursement for clinical laboratory testing, the prudent use of common diagnostic tests in patient care is increasingly important."

Partnership With Antimicrobial Stewardship Program Cuts C. Diff Testing

Implementation of a clinical review and preauthorization protocol led by antimicrobial stewardship program (ASP) can decrease inappropriate testing, according to a study published in the March issue *of Infection Control & Hospital Epidemiology*. Further, the multidisciplinary, multifaceted intervention reduced the hospital-onset (HO) C. difficile infection incidence rate (HO-CDI-IR) and antibiotic consumption.

Screening for C. difficile in asymptomatic patients is not recommended by consensus guidelines, in part because highly sensitive diagnostic technologies, such as nucleic acid amplification tests (NAATs) have a difficult time differentiating carriers from active infections. These false positives can drive unnecessary antibiotic treatment in asymptomatic patients.

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The researchers from Northwestern Memorial Hospital evaluated whether a diagnostic stewardship initiative consisting of ASP preauthorization paired with education was effective at reducing false-positive, HO-CDI among patients admitted between Oc.1, 2016, and April 30, 2018. Patients admitted to the stem cell transplant unit were not included in the intervention and served as a control group.

ASP preauthorization occurred for hospitalized patients for whom a computerized provider order entry order for an NAAT was placed and a specimen was sent for testing to the clinical microbiology laboratory. The intervention required the prescriber to attest that diarrhea has met CDI clinical criteria, to confirm no medication could cause the diarrhea, and confirm no recent C. diff polymerase chain reaction test had been ordered. Data were compared 33 months before and 19 months after implementation.

The researchers found that over the entire 52-month period, the mean facility-wide HO-CDI-IR was 7.8 per 10,000 PD. The mean HO-CDI-IR significantly decreased from baseline during the intervention, while HO-CDI-IR in the noninterventional control unit did not change.

"Our findings demonstrate that ASP preauthorization coupled with individual provider education can meaningfully reduce clinical false-positive NAAT results while also decreasing antibiotic overuse," conclude the authors led by **Alyssa B. Christensen**, Pharm.D., from Rosalind Franklin University in Chicago, Ill.

Takeaway: IT solutions are having an impact on infectious disease diagnostic stewardship. While system-wide benefits are seen, labs may notice decreased volumes post-implementation of these EHR-based solutions.

Technical Factors behind Discordant Results with Liquid Biopsies, *from page 1*

The need for a noninvasive test to monitor a patient's ongoing mutational status is fueling the integration of liquid biopsy testing into clinical practice. The U.S. Food and Drug Administration approved several plasma-based genotyping tests for clinical use, including tests that analyze hot spot mutations or specific genes such as EGFR and KRAS, as well as some gene panel tests—Oncomine (Thermo Fisher Scientific), Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets, and FoundationOne (Foundation Medicine).

Previous studies have shown substantial discordance of genomic results between tumor- and plasma-based results. This discordance has been attributed to low tumor content in plasma, limits of assay detection, and tumor heterogeneity.

The present study sent baseline plasma, tumor, and normal tissue samples from 24 patients to four circulating tumor DNA (ctDNA) sequencing vendors. Variant results were compared between the sources. The samples were considered "challenging" as they had limited ctDNA from early-stage cancers (lung, breast, ovary, and prostate cancers). The four laboratories were all CLIA-certified, commercial NGS laboratories. Vendor B had the smallest panel (approximately 20 genes), whereas the three other vendors had panels of 60 to 70 genes. Vendor names were blinded to encourage communication about laboratory and bioinformatic practices.

"The set of plasma samples analyzed allowed ctDNA NGS methods to be evaluated under challenging conditions … however, the approach may have resulted in a higher-than-expected discrepancy rate," explain the authors. "According to our results and those of others, application of ctDNA assays to early detection analyses may be confounded by FN and FP rates."

The researchers found that all vendor assays performed well above 10 percent variant allele frequency (VAF). However, below 1 percent VAF, most variant calls were discordant. These discordant calls were mainly the result of low-VAF calls, mutational biases, and novel somatic variants, the authors concluded. Additional, technical artifacts included background noise, bioinformatic filtering thresholds, and germline variant calls. The authors suggest that flagging and soft filtering for low-VAF calls, mutational biases, and novel somatic variants may reduce FPs.

Overall, positive predictive value (PPV) against tissue ranged from 36 percent to 80 percent. However, three vendors achieving a PPV of 100 percent, when limited to mutations called at an allelic fraction (AF) greater than 1 percent. For mutations called with an AF less than 1 percent, PPVs were as low as 17 percent.

"Variants detected at less than 1 percent AF are routinely reported by each vendor, and such sensitivity is advertised as a unique strength of plasma NGS assays," the authors note. "Fifty percent (22 of 44) of all true positive somatic variants had a VAF of less than 1 percent, underscoring the importance of analytically validated assays with sensitivity below 1 percent VAF."

Most FP calls were novel variants not found in somatic variant databases. The researchers examined raw data to better understand FN variant calls. One vendor called the PIK3CA H1047R variant, which was missed in another vendor's report even though it was present in that vendor's raw data. Despite similar number of reads for the two vendors, it was seemingly below one vendor's bioinformatics calling threshold. One vendor missed TP53 V143M due to elevated background noise in its assay, but the variant was present and reported by another vendor. In another instance, BRCA2

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125I8V was reported by one vendor at 20 percent VAF but was not reported by another vendor despite its presence in that vendor's raw data, possibly because of a bioinformatic filtering of suspected germline variants.

"Plasma cell-free DNA can be a scarce specimen, which makes paired analysis across multiple laboratories extremely challenging," writes lead author **Cloud Paweletz**, from Dana-Farber Cancer Institute in Boston, Mass., in an accompanying editorial. "Additional investment in validated reference materials could be one step toward establishing a reference point that laboratories can use to confirm the accuracy of their results, as well as a step toward improving the quality of testing for our patients with cancer."

Takeaway: Technical factors, including mutational bias, bioinformatics filtering, and assay sensitivity issues, contribute heavily to discordance in liquid biopsy test results more so than biological factors.