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INSIDE THIS ISSUE

Testing Trends: DNA Methylation Tests May Be the Next Game Changer in Low Cost, Noninvasive Cancer Testing

As liquid biopsies that analyze cell-free DNA (cfDNA) continue to gain traction in oncology, several companies are stepping up efforts to develop other noninvasive early cancer detection tests using similar biomarkers. One of the most promising approaches are tests that can detect abnormal DNA methylation associated with early-stage cancers. Here are some of the DNA methylation techniques in the pipeline based on the presentation of companies at the American Society of Clinical Oncology's (ASCO) virtual annual meeting in early June.

The Diagnostic Promise

Blood plasma contains cfDNA composed of fragmented DNA released by cells into the circulation, typically after the cell dies. If a patients have cancer, their plasma will include cfDNA released by tumor cells, aka, circulating tumor DNA (ctDNA). Methylation analysis involves chemically treating cfDNA to

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Inside the Diagnostics Industry: Infectious Disease Testing on Path to Steady Growth, Even Without COVID-19

From a pure business perspective, the pandemic has been a windfall for Abbott, Cepheid, PerkinElmer, Roche, Thermo Fisher Scientific and other COVID-19 testing companies. However, while the COVID-19 revenue windfalls of the past year are already starting to trail off, the long-term market for biomarker-based infectious disease testing is beyond sustainable. A new report from laboratory industry financial consulting firm Kalorama Institute says that the global market for such testing will reach nearly $15 billion in 2021. And that number does not even include COVID-19 testing.

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convert unmethylated cystosine to uracil and identifying changes in next generation sequencing.

Methylation patterns of ctDNA are consistent with the cells or tissues from whence they originate. Accordingly, ctDNA is a biomarker exhibiting cancer-specific and epigenetic aberrations which can be used as a surrogate source of tumor DNA in cancer diagnosis. And because methylation happens early in cancer development, ctDNA methylation patterns can be used to detect early-stage cancer.

Bluestar Genomics

During the ASCO meeting, San Diego-based Bluestar Genomics new data on a liquid biopsy assay it is developing for breast, colorectal, lung, ovarian and pancreatic cancer by tracking 5-hydroxymethylcytosine (5hmC) DNA modifications in a blood sample. Bluestar researchers isolated DNA from 176 fresh frozen tissues from stage I to IV cancer patients and collected cfDNA from the plasma of 783 non-cancer controls and 567 cancer patients. It then enriched for 5hmC using chemical labeling and sequenced the samples in normal and tumor tissues. After aligning the results to a reference genome to build feature sets of 5hmC patterns, the researchers identified specific and discrete gene-based features for both tumor and normal tissue. Finally, they applied a machine learning algorithm to the cfDNA dataset to spot a signature allowing for classification between non-cancer and cancer patients.

On a cancer-specific level with a specificity of 99 percent, the assay had a sensitivity of:

- 30 percent for breast cancer;
- 43 percent for colorectal cancer;
- 52 percent for lung cancer
- 57 percent for pancreatic cancer; and
- 75 percent for ovarian cancer.

Based on these results, Bluestar believes that the combined epigenomic and genomic profiles can distinguish between cancer and normal tissues and between different cancer types in asymptomatic high-risk individuals.

Avida Biomed

Fremont, California-based Avida Biomed, which has already developed a targeted methylation sequencing, or TMS, dual analysis assay called Point-N-Seq, presented data validation data for an updated version of the assay that incorporates both genomic and epigenomic analysis without sample splitting. The Avida research team used spike-in titrations of cancer cell line genomic DNA with known mutations and methylation profiles and achieved a detection level down to .003 percent of tumor DNA.

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First, the team integrated a colorectal adenocarcinoma TMS panel covering 560 methylation markers with a mutation panel with more than 350 hotspot mutations in 22 genes into the assay. Using plasma from a cohort of colorectal cancer patients, it detected cancer-specific methylation signals and oncogenic mutations in all samples.

In a cohort of stage I/II CRC patients, the team compared tumor-informed, personalized-mutation panels (100 SNVs) and tumor-independent colorectal cancer methylation panels for each patient. **Result:** The integrated assay achieved similar detection as the personalized tumor-informed approach, with an Area Under the Curve (AUC) factor of 0.91. In the pilot study performed on plasma of cancer patients at different stages, at 91 percent specificity, the assay had sensitivity of:

- 70 percent for stage I (75 patients);
- 94 percent for stage II (46 patients);
- 96 percent for stage III (24 patients); and
- 96 percent for stage IV (23 patients).

Based on these results, Avida believes the new TMS dual assay will have clinical and research application for early cancer detection, colorectal minimal residual disease (MRD) detection and patient monitoring.

3. Guardant Health

Guardant Health, which is challenging Exact Sciences for share of the early colorectal cancer and MRD detection market (See *DTET*, March 21, 2021), presented data on LUNAR-2, its developmental blood-based colorectal neoplasia diagnostic assay that assesses somatic mutations and methylation and fragmentation patterns in ctDNA to improve sensitivity for early-stage colorectal cancer detection. Guardant researcher used the LUNAR-2 assay to analyze plasma samples from 434 colorectal cancer patients with CRC prior to resection and 271 aged-matched controls. Training the assay on a separate 614-sample set, the Redwood, Ca., firm found:

- A 94 percent overall colorectal cancer specificity;
- An 88 percent sensitivity in stage I-II patients; and
- A 93 percent sensitivity in stage III patients.

There were no differences in sensitivity between asymptomatic and symptomatic colorectal cancer cohorts; higher cfDNA tumor fractions were found in the latter.

Guardant says it expects LUNAR-2 to achieve “meaningful performance in an average risk screening population.” To prove its hypothesis, the firm has launched a prospective registration study to evaluate the assay in an average colorectal cancer risk screening cohort.

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Takeaway

Liquid biopsy was a significant step forward for cancer treatment and the diagnostics industry. Now firms in that space are doubling down on developing new cfDNA-based technologies to further advance noninvasive early cancer detection. Tests using DNA methylation to detect ctDNA associated with specific tumors appears to be an extremely promising and exciting path forward.

FDA WATCH

First Ever Approval of COVID-19 Blood Spot Self-Collection & Molecular Test Pooling

The U.S. Food and Drug Administration (FDA) continues to step outside its traditional comfort zone in response to COVID-19 pandemic pressures. Among the most recent agency firsts is best seen as part of the current imperative to clear assays and collection kits for rapid and easy screening, including products that can be used in home settings. On April 6, the FDA crossed a new barrier by granting Emergency Use Authorization (EUA) for a SARS-CoV-2 antibody detection test used in dried blood spot samples collected at home.

The Symbiotica System

The product on the receiving end of the groundbreaking EUA was Symbiotica's COVID-19 Self-Collected Antibody Test System, a prescription-only test designed to detect immunoglobulin G against SARS-CoV-2 in dried blood spots obtained via fingerstick. The kit includes lancets that individuals can use to collect the blood samples themselves and mail to Symbiotica's California laboratory for analysis.

"The authorization of the first prescription use, home collection antibody test will play an important role in helping health care professionals identify individuals who have developed an adaptive immune response from a recent or prior COVID-19 infection," noted Jeff Shuren, director of the FDA's Center for Devices and Radiological Health, in a statement.

Molecular Test Pooling

Two weeks after clearing the Symbiotica system, the agency amended its guidelines to facilitate authorization for pooling by allowing molecular COVID-19 tests which have previously received EUA to be used with pooled samples performed to screen the asymptomatic as part of a "serial testing program," such as in a school or workplace setting. In other words,

specific clearance for the use is not required as long as the test developer self-certifies that it has validated the test for pooling and submits its validation data and pooling procedures.

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Here are some of the key new FDA EUAs and clearances announced in June:

New FDA Emergency Use Authorizations (EUAs) & Approvals

Manufacturer(s)	Product
Yale School of Public Health	Reissued EUA allowing use of SalivaDirect SARS-CoV-2 test with additional thermocyclers
LetsGetChecked	Reissued EUA allowing use of at-home COVID-19 sample collection kit for children as young as age 2
Amazon.com via STS Lab Holdco subsidiary	EUA for Amazon Real-Time RT-PCR DTC Test for Detecting SARS-CoV-2
Diabetomics	EUA for CovAb SARS-CoV-2 Ab Test
OraSure Technologies	EUA for InteliSwab COVID-19 Rapid Test for over-the-counter use
OraSure Technologies	EUA for InteliSwab COVID-19 Rapid Test Pro for professional use at point-of-care
OraSure Technologies	EUA for InteliSwab COVID-19 Rapid Test Rx for home use with a prescription
Sysmex America	Clearance for XN-10 Automated Hematology Analyzer with Blood Bank mode
Thermo Fisher Scientific	EUA for TaqPath COVID-19 Pooling Kit
Foundation Medicine	Accelerated approval for FoundationOne CDx to identify advanced cholangiocarcinoma patients with FGFR2 fusions or rearrangements who are eligible to receive newly approved BridgeBio Pharma and Helsinn Group's infigratinib (Truseltiq)
Qiagen	Approval for Therascreen KRAS RGQ PCR kit as companion diagnostic for newly approved Amgen's sotorasib (Lumakras) for previously treated, locally advanced, or metastatic non-small cell lung cancer patients whose tumors harbor a KRAS G12C mutation
Guardant Health	Approval for s Guardant360 CDx as companion diagnostic for newly approved Amgen's sotorasib (Lumakras) for previously treated, locally advanced, or metastatic non-small cell lung cancer patients whose tumors harbor a KRAS G12C mutation
DiaSorin	EUA for Liaison SARS-CoV-2 TrimericS IgG chemiluminescent immunoassay
Salofa	EUA for Sienna-Clarity COVID-19 Antigen Rapid Test Cassette
Harvard University	EUA for point of care immunoassay Quaeris SARS-CoV-2 Assay
NowDiagnostics	EUA for AdexusDx COVID-19 Test lateral flow immunoassay

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FDA Watch, *from page 5*

Manufacturer(s)	Product
Janssen Pharmaceutical	Accelerated approval for EGFR-MET bispecific antibody amivantamab (Rybrevant) to treat EGFR exon 20-mutated NSCLC
Phosphorus Diagnostics	EUA for direct-to-consumer version of saliva-based SARS-CoV-2 assay and use of at-home sample collection kit with test

Emerging Tests: New Studies Support Viability of Reliable COVID-19 Lateral Flow-Based Home Testing Device

As deaths and case rates fall, the focus of COVID-19 testing will continue to shift from diagnosing patients to screening the asymptomatic. This will fuel the demand for products that can deliver results accurately, quickly and at the point of care. The fly in the ointment is that current SARS-CoV-2 antigen tests that meet these requirements are nowhere near as accurate as the tests used for diagnosis applications. However, new research suggests that an alternative technology support the viability of a more accurate rapid testing solution: lateral flow assays.

The Diagnostic Challenge

Polymerase chain reaction (PCR) tests that detect SARS-CoV-2 nucleic acids from the virus' RNA were the first tests used to identify patients with COVID-19. And they remain the gold standard in accuracy with false negative rates ranging less then 5 percent, depending primarily on the sampling site, sample type and stage of infection). The downside of PCR tests is that the RNA from the sample must be amplified and converted into DNA for testing, operations that must be performed at a CLIA-certified laboratory off the site.

Rapid antigen tests use antibodies that the body produces to fight the SARS-CoV-2 virus to detect specific parts of the viral particle itself. Antigen tests are the yin to PCR tests' yang: They deliver rapid results at the point of care but they are relatively lacking in accuracy. The biggest problem with antigen tests is their susceptibility to false negatives. Using them for screening purposes thus entails the risk that asymptomatic individuals with infections will be allowed to enter schools, workplaces, airplanes, ballgames and other settings where they may spread the virus to others.

The optimal solution for screening would be to somehow combine the scalability and mobility of antigen tests with something approaching

the accuracy of PCR testing. Such a solution would also be useful as the opening stage in the diagnosis and treatment process, much like current home pregnancy tests where users who test positive would call their physicians for confirmatory tests and initiate precautionary measures like self-isolation.

The Potential of Lateral Flow Technology for COVID-19 Detection

Lateral flow tests (LFTs) are simple devices that use immunoassay technology to detect the presence of a target substance in a urine, blood, saliva or other liquid sample. LFTs operate along the same principles as current enzyme-linked immunosorbent assays (ELISA). The tests run the liquid sample along the surface of a pad with reactive molecules that show a visual positive or negative result. The pads are based on a series of capillary beds, each of which pads the capacity to transport fluid spontaneously.

Because they do not rely on specialized and costly equipment, LFTs are well suited for home use. One of the most common applications of LFT technology for medical use is home pregnancy testing capable of detecting hormones in urine that are associated with pregnancy in the range of five to 30 minutes.

Can LFT Be Trusted for COVID-19?

But while LFT technology is cheap and fast, its capability for use in reliably detecting COVID-19 remains unproven. The lack of test sensitivity makes LTFs prone to produce false negative results. Last September, the World Health Organization warned that "very few" LFTs had undergone "stringent" regulatory procedures. In a mass-testing pilot carried out in the UK at the end of 2020 in Liverpool, tests made by Innova Medical Group missed 60 percent of asymptomatic cases.

Identifying the Cause of False Negatives: The King's College Study

The good news is that recent research suggests that it is possible to cost-effectively produce more sensitive LFTs for use in COVID-19 testing. One notable example is a new study published in the journal ACS Materials and Interfaces that explains why LFTs produce such large numbers of false negatives and what modifications could be made to improve accuracy.

Researchers from King's College London used X-ray fluorescence imaging from Diamond Light Source Trust, a national science facility to image how the virus interacts with the tests. They found that the underlying technology of many LFTs is highly accurate and theoretically capable of detecting trace amounts of the SARS-CoV-2 virus. The problems, they concluded, stem not from the test technology but limitations in the technology used to communicate the result of the test, i.e., the read-out. They then lay out a series of relatively simple technical modifications that could be made to potentially improve the performance of LFTs.

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Improving LFT Sensitivity: The Berkeley Lab Study

And now a new study from Lawrence Berkeley National Laboratory (Berkeley Lab) suggests that it is possible to develop a highly sensitive LFT for COVID-19. The assay would be done on mucus samples taken from the nose or throat using a swab, which would be dipped in a tube containing a solution to dilute the sample, and then placed at one end of a porous strip in a test cartridge. As the sample is pulled along the strip via capillary action it would draw pairs of rigid antibodies designed to recognize and bind onto SARS-CoV-2 antigens. As with a home pregnancy test, a colored band would appear on the strip if the test subject has a COVID-19 infection in 15 minutes.

The researchers, led by Michael Hammel and Curtis D. Hodge, used small angle X-ray scattering (SAXS) performed at Berkeley Lab's Advanced Light Source (ALS) to examine about 20 antibody-antigen interactions. They found that a particular pair of monoclonal antibodies bound to the nucleocapsid protein very strongly, in part due to the antibodies' rigidity. "The combination of the two rigid antibodies was also observed to increase networking—a process in which multiple antibodies bound to the same antigen at different sites form larger clumps or 'networks,'" Hodge explained.

It has long been understood that antibody networking and high binding stability improve LFT sensitivity. But studying the physical dynamics of antibody-antigen pairs to find the most effective antibodies is very difficult with traditional imaging techniques, which require the molecules to be stabilized or crystallized. The SAXS technique developed by Hammel and his colleagues allows scientists to examine antibodies and antigens in their natural state, i.e., when moving freely in a liquid.

"We showed that we can rapidly identify new antibody-antigen pairs that result in a more sensitive detection assay," said Hammel. "This technique could be applied to hundreds of antibodies in a short amount of time to identify the most suitable antibodies to achieve as-of-yet unattained sensitivity of antibody-based diagnostics, which are key for early diagnosis of SARS-CoV-2 as well as other pathogens."

Takeaway

While LFTs offer significant advantages in costs, ease of use and speed, many in the scientific community have warned that they lack the sensitivity necessary for COVID-19 screening. And this may be true. However, many powerful actors, including the British government, have made LFT devices modeled after home pregnancy tests a centerpiece of their COVID-19 response strategy. And now scientific evidence is emerging to suggest that this might prove to be a very wise decision.

Emerging Tests: Simple Urine Test May Be Capable of Predicting COVID-19 Case Severity

Analyzing a COVID-19 patient's urine sample can help treatment providers predict how severe the case will be. That is the finding of a new study created by researchers from Detroit-based Wayne State University. Such an assay would be a useful tool for screening hospital patients and preserving treatment resources.

The Diagnostic Challenge

COVID-19 affects different people different ways. Some people experience no symptoms at all. But others may experience pneumonia with multiple organ failure, and death. Early identification of critical patients requiring more aggressive intervention could prove instrumental in reducing COVID-19 deaths.

One of the most promising leads in that regard is the recognition that in many severe COVID-19 cases, patients produce cytokine proteins at elevated levels. These so-called "cytokine storms" cause inflammation in multiple organs, including the heart, lungs and brain. Accordingly, urine tests that measure cytokine levels may be effective in predicting case severity. Such tests are currently used for screening urinary tract infections, interstitial cystitis and other illnesses.

The Wayne State Study

With this in mind, the Wayne State research team set out to determine whether COVID-19 biomarkers were capable of predicting which individuals will develop cytokine storms. The study compared urine cytokine levels in 17 patients with confirmed COVID-19 cases and 10 patients without the infection.

The study results, which were first revealed during the annual meeting of the American Physiological Society, found that the cytokine levels of patients who had the virus were "significantly elevated," specifically:

- Growth-regulated oncogene; and
- Interleukin-6 (IL-6).

The most highly elevated urinary cytokine levels were found in patients with pre-existing chronic health conditions such as high blood pressure and diabetes. People with these health conditions are considered to be at higher risk of severe illness from COVID-19, the researchers concluded.

In addition, the researchers found that in all cases, cytokine levels retreated to normal ranges once the infection resolves.

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Emerging Tests: Simple Urine Test May Be Capable of Predicting COVID-19 Case Severity, *from page 9*

"We have observed, albeit in a small cohort of patients that we examined, that the urinary cytokine levels increase as the infection progresses, and that they decrease as the infection resolves," study co-author and Wayne State assistant professor of research Dragana Komnenov told a UPI reporter in an email. "This suggests that the urinary cytokine signature could potentially have diagnostic and-or prognostic value."

Takeaway

The pandemic is winding down but people are still dying from the SARS-CoV-2 virus. This dynamic is likely to accelerate efforts to develop COVID-19 diagnostics not simply to detect infections but also generate results to support treatment decisions. The hope is that the Wayne State study will translate into a regular process for severity screening of COVID-patients.

Another promising effort in this direction is a study from scientists at the University Medical Center Goettingen in Germany finding that leukocytes and albumin in urine may also be a reliable biomarker for use in predicting the severity of COVID-19 infection.

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The Biomarker Infectious Disease Test Market

Biomarkers are biological or biochemical molecules, genetic changes and other measurable characteristics that are used for diagnostic, treatment and medical research applications, including identifying diseases, prognosis, evaluating genetic risks for certain conditions, disease monitoring, determining appropriate treatment options, etc. Biomarkers used to detect infectious agents include proteins (antigens), antibodies produced in response to the agents, genomic markers, etc.

Kalorama is one of the best sources of data and analysis profiling different segments of the laboratory testing market. Its new report "World Market for Diagnostic Biomarkers," predicts that global sales of biomarker-based infectious disease tests, excluding COVID-19 tests, will reach $14.8 billion in 2021. Kalorama also projects compound annual revenue growth of 5.8 percent per year from 2021 to 2027. Of course, those numbers become much bigger when the COVID-19 wildcard is included. These data are based on the three major types of biomarker-based infectious disease tests, in order of how fast they are expected to grow in volume and market share between now and 2027:

1. Molecular Tests

The segment of the biomarker infectious disease testing market that Kalorama expects to grow the fastest is molecular testing, including polymerase chain reaction (PCR), microarrays and other assays that detect nucleic acids, DNA, RNA and genes associated with particular pathogens. Rapidity of growth will be driven by the fact that molecular tests are more accurate than antigen and antibody tests, which makes them more likely to be used for more complex infectious disease testing applications and treatment settings requiring a more definitive diagnosis.

While it is not part of the Kalorama growth analysis, this is the same pattern that has played out with COVID-19 testing. In addition to COVID, molecular tests are commonly used to detect HPV, *Chlamydia trachomatis/Neisseria gonorrhoea* (CT/NG), and tuberculosis. Another growing application of molecular tests is the detection of resistance genes linked to healthcare-associated infections and disease-resistant pathogens.

2. Rapid Immunoassays

The Kalorama report predicts moderate growth for rapid immunoassays based on lateral flow immunochromatographic techniques capable of detecting pathogens in both medical treatment home and other point-of-care settings. There are currently rapid immunoassays for bacterial

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vaginosis (BV), chlamydia, dengue, Ebola, HIV, influenza, Legionnaires' disease, malaria, rotavirus, respiratory syncytial virus (RSV), group A *Streptococcus, Treponema pallidum*, and *Trichomonas vaginalis*.

3. Laboratory Immunoassays

Kalorama predicts slower growth for laboratory immunoassays, currently the largest segment of the biomarker infectious disease test market and which is expected to account for over 60 percent of global sales over the long term. The strength of this segment is the capacity of these assays, particularly enzyme-linked immunoassays, to detect antibodies and antigens associated with a wide range of common pathogens, including influenza, pneumonia, *Clostridium difficile* (C. diff), hepatitis C, HIV, herpes simplex virus (HSV) and Lyme disease.

Takeaway

Infectious disease testing based on biomarkers was on a steady arc well before the pandemic hit. The Kalorama report is useful in re-establishing the larger context without the unpredictable COVID-19 factor. What we do know is that COVID-19 will lift all segments. However, the pattern of growth, at least in the short term, will likely run against the grain of the larger infectious disease market, with laboratory immunoassays growing the fastest due to the need for rapid tests that can be used to screen the asymptomatic at home and other non-treatment settings.