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Can Colorectal Screening Incorporate Adherence into Guidelines?

The U.S. Preventive Services Task Force's (USPSTF's) draft recommendations for colorectal cancer (CRC) screening acknowledge there are several viable screening options that can accurately detect early-stage CRC, including annual fecal immunochemical test (FIT) or high-sensitivity, guaiac-based fecal occult blood test (gFOBT); annual FIT plus flexible sigmoidoscopy every 10 years; or colonoscopy every 10 years. Yet, the USPSTF acknowledges that test characteristics are determined from studies of one-time use, rather than repeated screening over time, as would be seen in real-world application of the recommendations. This raises concerns about screening accuracy in real-world settings, where adherence rates are low.

"The important practical issue is whether an annual FOBT should continue to be recommended in current U.S. guidelines without further qualification,

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Is HCV Birth Cohort Testing Broad Enough?

Hepatitis C virus (HCV) infection is highly prevalent in patients seeking care in urban emergency rooms, according to two separate studies that each demonstrate a prevalence of approximately 14 percent. In both studies published in the May issue of *Clinical Infectious Diseases*, roughly one-third of positive cases were previously undiagnosed and more than a quarter of these HCV-positive cases would have remained undiagnosed using current screening strategies, causing the authors to call for expanding birth cohort-based screening criteria.

Current screening strategies for HCV detection rely on the U.S. Centers for Disease Control and Prevention (CDC) birth cohort screening recommendations—one-time HCV testing in those born from 1945 to 1965—in combination with targeted risk-based testing. Given that emergency departments serve as a safety net for underserved patients that may be at high risk for HCV because of HIV status and/or intravenous drug use (IDU), and that emergency departments have shown some success as an HIV screening venue, they may be a strategic partner for expanding national HCV screening.

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when it has been known for decades that adherence to a program of annual FOBT testing is low, resulting in poor effectiveness of the overall screening strategy," writes co-author Sidney Winawer, M.D., from Memorial Sloan Kettering Cancer Center in New York, in a viewpoint published May 17 in the *Journal of the American Medical Association*.

> *"In order for colorectal cancer screening programs to be successful in reducing deaths from the disease, they need to involve more than just the screening method in isolation."*
> —USPSTF

The USPSTF draft statement, released in October 2015, updates previous recommendations published in 2008. Over that time period, an additional 95 new studies were published and considered, including 24 studies assessing the impact of screening on CRC incidence and mortality, 19 new studies evaluating the diagnostic accuracy of screening tests, and 70 new studies that evaluated harms. Yet, no comparative studies (head-to-head studies between screening methods) were conducted that demonstrated one recommended screening strategy to be more effective than others.

Data shows though, that colonoscopy remains the most commonly used CRC screening test in the United States (more than 60 percent), while stool tests account for about 10 percent. It is widely known that CRC screening adherence is suboptimal, although improving. Recent U.S. estimates show that the overall proportion of adults "up to date" on CRC screening increased from 54 percent in 2002 to 65 percent in 2010, but nearly one-third of adults remain "never screened." Retesting adherence rates are higher in clinical trials and organized health plans (i.e., Veterans Administration [VA]), but even still, rates of annual retesting over four years remain as low as 14 percent, according to a VA study.

"In order for colorectal cancer screening programs to be successful in reducing deaths from the disease, they need to involve more than just the screening method in isolation. Screening is a package or cascade of activities that must occur in concert, cohesively, and in an organized way for benefits to be realized," writes the USPSTF in its draft recommendation.

The recommendations do address effective implementation strategies that have been demonstrated to increase appropriate use of CRC screening, including the use of clinician and client reminder systems, the use of small media (videos, letters, and brochures), reducing structural barriers to screening (time or distance to the screening delivery setting), and providing clinician assessment and feedback about screening rates."

"The question therefore becomes how best to recommend FOBT in CRC screening guidelines. Should FIT be offered with a more careful consideration of its retesting adherence?" writes Winawer and colleagues. "The FIT retesting adherence effectiveness relationship should be carefully considered in future CRC screening guidelines to maximize the effectiveness of a frequently used test. ... This would provide evidence-based guidelines with a critical pragmatic and reality-driven component."

Takeaway: While several CRC screening strategies appear effective based on one-time trials, future guidelines may need to address the effect that lack of adherence to testing strategies has on effectiveness.

USPSTF CRC Draft Recommendations

The USPSTF's October 2015 draft recommendation statement on CRC screening recommends colonoscopy every 10 years, flexible sigmoidoscopy every 10 years with annual FIT, or annual FIT or high-sensitivity gFOBT screening.

Large Panels Complicate Results Reporting for Breast Cancer Risk

Increasing the number of genes evaluated in individuals undergoing assessment for breast or ovarian cancer risk through large panels or whole exome sequencing (WES) does not increase the rate of molecular diagnosis, but rather, complicates reporting with a "substantial burden" of incidental and uncertain results, according to a study published May 5 in the *American Journal of Human Genetics*. The study also found clinical utility in the American College of Medical Genetics and Genomics (ACMG) variant-classification guidelines.

The plummeting cost of sequencing and identification of an increasing number of potential breast-cancer-susceptibility variants have driven interest in the clinical use of large, multi-gene panels (sometimes with 100-plus genes) and WES to assess genetic cancer risk. However, the expanded sets of genes quarried have varying levels of evidence to support their association with cancer susceptibility and the large gene sets complicate results reporting because of incidental findings.

"The high concordance of variant calls with LSDBs and Clinvar suggests the clinical utility of variant classification based on ACMG guidelines coupled with expert review."

— Katherine Nathanson, M.D.

Researchers from the SIMPLEXO (Simplifying Complex Exomes) consortium evaluated the ability of a variant-classification methodology based ACMG guidelines to define the rate of mutations and variants of uncertain significance (VUS) in 180 medically relevant genes in individuals who met guidelines for hereditary cancer risk evaluation. WES was performed in 404 individuals (from 253 families).

The researchers found 1,640 unique, non-silent exonic germline variants in 167 of the 180 genes. For genes with at least one variant identified, on average, 10 variants per gene were found. Individuals had on average 97 nonsynonymous variants and small insertion or deletion variants. Potentially clinically actionable variants, which included likely pathogenic or pathogenic calls, were 95 percent concordant with locus-specific databases (LSDB) and Clinvar, but absolute call concordance was lower for all genes, compared to those that were potentially actionable.

The vast majority of individuals tested (95 percent) had at least one variant of unknown significance (VUS), but the rate varied substantially from 12 percent for the well-established breast cancer susceptibility genes versus 78 percent when additional genes associated with autosomal-dominant (AD) cancer susceptibility were analyzed. VUS rates were also high for the non-cancer-associated genes (77 percent of families) and genes associated with autosomal-recessive cancer susceptibility (68 percent).

"The high concordance of variant calls with LSDBs and Clinvar suggests the clinical utility of variant classification based on ACMG guidelines coupled with expert review," writes senior author Katherine Nathanson, M.D., from University of Pennsylvania in Philadelphia. "Our results strongly support the view that additional research studies are needed to resolve VUSs and understand the implications of incidental and/or unexpected results obtained from genetic testing for the assessment of cancer susceptibility. Our data do not support the use of medical exome or WES for evaluation of cancer susceptibility in individuals at high risk for breast and/or ovarian cancer at this time."

Takeaway: Increasing the number of genes assayed for testing for breast and ovarian cancer susceptibility has minimal clinical impact and leads to increasing clinical complexity as a result of increased identification of VUS.

Inside The Diagnostics Industry

Urine Becoming Preferred Zika Sample as Testing Industry Prepares for Summer Mosquito Season

Diagnosing Zika virus is challenging. While diagnosis previously relied on serology, it is difficult to differentiate Zika antibodies from other closely related viruses, including dengue. As a result, the World Health Organization (WHO) has encouraged test developers to move away from serology-based assays towards nucleic acid-based assays that are more specific and sensitive, if performed during the acute phase of infection. But, new evidence shows that the sensitivity of nucleic acid testing can be impacted by sample type.

Test developers around the world are working to quickly deploy tests for surveillance, clinical diagnosis and monitoring, and blood donation screening. *Diagnostic Testing and Emerging Technologies (DTET)* undertook a sampling of evolving testing recommendations, federal efforts to speed diagnostic development, and industry's progress on bringing tests to market as the Southern sections of the United States brace for mosquito season and the possibility of local Zika transmission.

Testing Recommendations

The U.S. Centers for Disease Control and Prevention (CDC) recommends testing for potentially exposed persons with signs or symptoms consistent with Zika virus infection and for asymptomatic pregnant women within 12 weeks of exposure.

From Jan. 3, 2016 to March 5, 2016, Zika virus testing was performed for 4,534 people in the United States who traveled to or moved from areas with active Zika virus transmission, with pregnant women accounting for nearly three-quarters of those tested (73.6 percent), according to a report from the CDC's Epidemic Intelligence Service published April 22 in *Morbidity and Mortality Weekly Report* (*MMWR*). The authors say that while currently the likelihood of Zika infection among asymptomatic persons in the United States with a travel-related exposure history is low (less than one percent among pregnant women tested in the United States), providers should continue to offer testing to asymptomatic pregnant women with potential exposure due to the potential for adverse pregnancy and infant outcomes with perinatal Zika infection.

Preferred Sample Guidance

As more is understood about the biology of the Zika virus, modes of testing are changing. Significant development efforts are underway for improved molecular and serologic assays for detection of Zika infection. In addition to clinical diagnostics, assays are in development for blood, organ, and tissue donor screening, given the recent reports of several probable cases of transfusion transmission in Brazil.

"Nucleic acid testing is moving forward well with extremely high sensitivity and throughput, development efforts are very successful," Michael Busch, M.D., Ph.D., co-director of the Blood Systems Research Institute in San Francisco, tells *DTET*. "On the serology size there are fundamental challenges, biological challenges, in populations with a history of dengue. In general there is good progress on the development of laboratory-based assays."

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In an interim guidance published May 13 in *MMWR*, the CDC says that real-time reverse transcription–polymerase chain reaction (rRT-PCR) is the preferred test for Zika virus infection because of its rapid turnaround and its high specificity. However, given emerging information, urine samples may be preferable to serum depending on the length of time from onset of symptoms.

Zika virus RNA is unlikely to be detected in serum after the first week of illness, but Zika virus RNA can be detected in urine for at least two weeks after onset of symptoms, the CDC says. Therefore, CDC recommends that Zika virus rRT-PCR be performed on urine collected less than 14 days after onset of symptoms in suspected patients and that rRT-PCR urine testing be performed in conjunction with serum testing if specimens are collected less than seven days after symptom onset.

"Urine might be the preferred specimen type to identify acute Zika virus disease."

— Andrea M. Bingham, Ph.D.

This recommendation was based on findings of a Florida Department of Health study of multiple specimen types from persons with suspected Zika virus disease. Test results were classified by specimen type and number of days after symptom onset to determine the most sensitive and efficient testing algorithm for acute Zika infection. Results were published May 10 in *MMWR*.

Zika virus RT-PCR was performed at the state's public health laboratory using a laboratory-developed test based on a previously published protocol using two RT-PCR targets. Serologic testing was performed on all serum specimens. Case definition criteria for Zika virus disease was based on serology and required Zika virus–specific IgM antibodies and no dengue virus–specific IgM antibodies in serum or cerebrospinal fluid samples. Urine and saliva RT-PCR tests were only used for surveillance purposes, while serum analysis was used for diagnostic purposes.

Urine specimens were collected from 70 patients with suspected Zika virus disease from zero to 20 days after symptom onset. The vast majority (93 percent) tested positive for Zika virus RNA using RT-PCR. For urine specimens collected from persons within five days of symptom onset, 95 percent (52 of 55) tested positive by RT-PCR compared to only 56 percent (31 of 55) of serum specimens collected on the same date. For specimens collected more than five days after symptom onset, 82 percent (9 of 11) of urine specimens tested positive by RT-PCR, compared to none of the serum specimens. Viral RNA was detectable in urine as early as the first day of symptoms and as late as 20 days after onset of symptoms.

Zika virus IgM antibodies were detectable in 40 percent (22 of 55) of the serum specimens, including two specimens collected one day after symptom onset. Among the 31 cases in which urine specimens tested positive by RT-PCR, but serum specimens tested negative, Zika virus IgM antibody was detected in 74 percent of the serum samples.

"Results of testing conducted at Florida Department of Health Bureau of Public Health Laboratories suggest that urine might be the preferred specimen type to identify acute Zika virus disease," writes lead author Andrea M. Bingham, Ph.D.

Expanded Funding for Testing, Test Development

Federal agencies are accelerating efforts to combat the Zika epidemic on multiple fronts.

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Increasing Sample Access - Industry cites a lack of access to positive Zika blood samples as a significant barrier to advancing test development. To speed the development of diagnostic tests for Zika virus infection, the U.S. Department of Health and Human Services' Office of the Assistant Secretary for Preparedness and Response (ASPR) is supporting the collection of blood samples from people in the continental United States and Puerto Rico who have been infected with Zika virus. Under a six-month, $692,000 project funded by ASPR's Biomedical Advanced Research and Development Authority, Clinical Research Management (Hinckley, Ohio), will collect blood samples from people with confirmed Zika virus infection, in coordination with state and local health departments and the CDC. These samples will be collected and made available to diagnostic companies for use in validating the performance of their tests.

Military Enhances Testing, Surveillance - The Armed Forces Health Surveillance Branch allocated $1.76 million in additional funding in mid-May to 10 Department of Defense (DoD) laboratories to enhance their Zika virus surveillance efforts worldwide. (This is in addition to the $51 million to support a range of emerging infectious disease surveillance programs already allocated this fiscal year to network partners.) The enhanced Zika virus surveillance will involve 10 projects in 18 countries and territories by four lab partners based in the United States and five located overseas. DoD labs will use the Zika money for three kinds of surveillance studies: retrospective examination for Zika virus exposure among DoD personnel through serum repository samples; expansion of clinic-based surveillance for Zika virus disease among DoD and civilian populations with febrile disease in California, Arizona and Texas; and expanded testing for Zika virus in mosquitoes in the Caribbean, East Africa, and Southeast Asia.

FDA's First EUA for a RT-PCR Zika Assay

The U.S. Food and Drug Administration (FDA) granted Emergency Use Authorization (EUA) for multiple tests to detect patients who have been infected with Zika virus. The first RT-PCR assay granted EUA was the CDC's Trioplex Real-time RT-PCR Assay for the qualitative detection and differentiation of RNA from Zika virus, dengue virus, and chikungunya virus. The test is intended for use with human sera or cerebrospinal fluid (collected alongside a patient-matched serum specimen), and for the qualitative detection of Zika virus RNA in urine and amniotic fluid (each collected alongside a patient-matched serum specimen). The FDA previously gave EUA to the CDC's Zika Immunoglobulin M (IgM) Antibody Capture Enzyme-Linked Immunosorbent Assay.

Sampling of Test Development Efforts

Below is a sampling of industry test development efforts.

Clinical Testing Devices

Tetracore (Rockville, Md.) deployed its Cirrus DX point of care molecular device (Tetracore T-COR 8 instrument and the Collect-to-Test cartridge) for the detection of Zika, dengue, and chikungunya viruses in collaboration with the Naval Infectious Diseases Diagnostic Laboratory on the island of Grenada in late May. The T-COR 8 instrument can run up to eight samples at a time and has a built-in battery that can run the instrument up to four hours without power. The proprietary collection device enables samples to go directly from the finger stick into the C2T cartridge without additional processing. This is the first time the system is being used for clinical diagnostics. Previously, the company says, the Cirrus DX solution was used to detect bio-threat agents and for veterinary testing. The system is expected to be deployed on other islands during the summer, enabling sharing of information about

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the spread of Zika in the region, through the system's connectivity capability that allows instrument access from anywhere in the world on a secure network.

altona Diagnostics (Germany) was granted EUA by the FDA in mid-May for its RealStar Zika Virus RT-PCR Kit, allowing the test to be used by CLIA High Complexity Laboratories. The molecular diagnostic tool can provide in vitro qualitative detection of RNA from the Zika virus in human serum or urine (collected alongside a patient-matched serum specimen) from individuals meeting CDC Zika virus clinical (symptoms) or epidemiologic (travel) criteria for testing. The kit can be used on multiple platforms.

Rheonix (Ithaca, N.Y.), in collaboration with New York University College of Dentistry (NYUCD), received funding to develop a rapid diagnostic for Zika virus infection. The $656,414 award supplements an existing Small Business Innovation Research grant to develop a fully automated screening and self-confirming assay to simultaneously detect and confirm the presence of Zika virus in a single, small sample of saliva or blood. The proposed approach is based upon previous success in which the Rheonix-NYUCD team developed a dual assay for the simultaneous detection of HIV antibodies and viral RNA in a single specimen. Richard Montagna, Ph.D., senior vice president for scientific and clinical affairs, tells *DTET* that the dual-assay, fully automated system can pick up early infections with viral RNA or later infections with antibodies, but that the company is still ensuring the assay can be adapted from HIV to Zika.

First Commercial Laboratory Clearance

Quest Diagnostics (Madison, N.J.) received FDA EUA for the Zika Virus RNA Qualitative Real-Time RT-PCR test at the end of April. The emergency clearance from the FDA is the first for a test developed by a commercial laboratory in the United States. The molecular test is intended for the qualitative detection of RNA from the Zika virus in human serum specimens. Quest Diagnostics revealed plans to make the new test broadly available to physicians for patient testing, including in Puerto Rico, early in May.

Investigational Approval for Blood Screening

Roche was granted approval to initiate collection and testing of blood samples for screening with the cobas Zika assay under an Investigational New Drug Application (IND) protocol. The cobas Zika test uses the cobas 6800/8800 Systems, to qualitatively detect Zika virus RNA in plasma specimens from human blood donors. Initially, the cobas Zika test will be deployed to screen blood donations collected locally in Puerto Rico, enabling the reinstatement of the blood services in Puerto Rico and reducing the reliance of blood importation from other areas in the United States. The second stage of deployment for the cobas Zika test will prepare for screening of blood donations collected by blood services in the southern United States, which could be impacted by the domestic spread in the virus during mosquito season.

Takeaway: As the Southern parts of the United States brace for summer mosquito season, the federal government and industry are trying to accelerate the readiness of clinical diagnostic and blood screening tests for Zika detection.

Is HCV Birth Cohort Testing Broad Enough?, *Continued from bottom of p.1*

"While we cannot know with certainty the extent to which our findings are generalizable to other emergency departments, we suggest that undiagnosed HCV is likely to be endemic in the emergency department populations of all but the smallest and most rural centers," writes lead author Michael Lyons, M.D., from University of Cincinnati (Ohio) in one of the studies. "As is the case with HIV, emergency departments are likely to provide a uniquely high level of access to populations with undiagnosed HCV who are in need of treatment."

In the Cincinnati study, the researchers used samples from a repository of 924 emergency department patients, who consented and provided and self-reported information (2008 to 2009) as participants for an HIV prevalence study. Testing relied upon an enzyme-linked immunosorbent antibody assay and viral RNA testing (Qiagen), followed by real-time reverse transcription polymerase chain reaction (Bio-Rad).

"As is the case with HIV, emergency departments are likely to provide a uniquely high level of access to populations with undiagnosed HCV who are in need of treatment."

— Michael Lyons, M.D.

The researchers detected HCV antibody in 128 of 924 (14 percent) of samples, with 44 of these (34 percent) from patients self-reporting a history of HCV or hepatitis of unknown type. In total, 103 of the 128 antibody-positive samples (81 percent) were RNA positive. Fully implementing birth cohort screening for HCV antibody would have missed 36 of 128 (28 percent) of cases with detectable antibody and 26 of 105 (25 percent) of those with replicative HCV infection.

Similarly, the second study, from Johns Hopkins Hospital Emergency Department (Baltimore, Md.), found that 25 percent of undocumented HCV infections would be missed using CDC guidelines.

The Johns Hopkins researchers conducted an eight-week seroprevalence study (2013) in patients with excess blood collected for clinical purposes. Demographic and clinical information, including documented HCV infection, was determined from electronic medical records. Testing used an HCV enzyme immunoassay (GreenCross Life Science) and HCV RNA was quantified (Abbott Laboratories) on 100 randomly selected samples of HCV antibody-positive samples.

The study showed that of 4,713 eligible patients, 652 (13.8 percent) were HCV antibody positive. Of these, 204 (31.3 percent) had undocumented HCV infection (4.3 percent of all tested). Among the 204 patients with undocumented HCV infection, 63 percent were in the 1945-1965 birth cohort, 22 percent were IDU, and 5 percent had known HIV infection, meaning that of patients with undocumented infections, 48.5 percent would have been diagnosed based on birth cohort testing, 26.5 percent would be identified by risk-based testing, and 25.0 percent would have been missed.

"Given an estimated 7,727 unique HCV antibody-positive patients attending the emergency department in a one-year period … there would be approximately 2,419 patients with undocumented infection," write the authors led by Yu-Hsiang Hsieh, Ph.D., from Johns Hopkins. "However, 605 patients (526 with chronic infection) would be missed in an emergency department-based HCV testing program using current CDC testing recommendations."

Takeaway: Given the high prevalence of HCV infection seen in urban emergency departments, expanding the target population of one-time HCV age-based screening may be appropriate.

Rapid, Panel Viral Testing May Be Cost Effective in Hospitals, Less So in Outpatient Settings

Panel-based diagnostics for respiratory viral (RV) infections may be cost-effective in hospital settings, according to several abstracts presented at the Clinical Virology Symposium (May 19-22; Daytona Beach, Fla.). Population-specific implementation can yield improved patient outcomes and cost savings in the hospital's emergency department and intensive care unit (ICU), but such comprehensive panels may not be cost effective in outpatient settings.

"Although random access molecular methods tend to be more costly than batch molecular assays, improved outcomes in certain populations, such as ICU, may warrant their use."

— Raquel Martinez, Ph.D.

Researchers from Geisinger Health System (Danville, Penn.) assessed the impact of rapid results of non-batched respiratory virus assays on downstream patient outcomes in the ICU. Geisinger performs multiplex RV testing prior to all admissions. From Nov. 1, 2010 to Mar 19, 2012, batch molecular RV testing (pre-intervention; n=278) was performed once per day for all inpatients (Luminex Corporation). From Mar. 19, 2012 to Apr. 30, 2014, this testing switched to a first-in first-out (FIFO), random access basis (post-implementation; n=462) with priority for ICU and emergency department orders (BioFire Diagnostics).

Post-implementation there were significant improvements in 28-day mortality, length of stay (LOS), ICU days, ventilator days, antimicrobial (including viral and bacterial) utilization, laboratory test utilization, and total cost. The mean collect to report time (CTR) dropped by 30.5 hours for the post-implementation period. This reduction in CTR was associated with significantly improved mortality when results were reported in less than seven hours. For patients with negative respiratory virus results, mean ICU stay, mean overall LOS, and mean total cost decreased by 3.3 days, 1.9 days, and $8,082.60, respectively.

"Although random access molecular methods tend to be more costly than batch molecular assays, improved outcomes in certain populations, such as ICU, may warrant their use," writes Raquel Martinez, Ph.D. "Additionally, this study demonstrated that 28 day mortality significantly increased when results were reported in more than seven hours. ... Attention to CTR appears to be an important quality indicator. ... Although a FIFO workflow may not be feasible for some laboratories, it may be possible to implement batch testing to achieve similar CTR."

Another study, from University Medical Center Groningen in the Netherlands, found that implementing rapid diagnostic testing for respiratory viral infections in the emergency department showed value not just in positive cases, but also in patients that tested negative. The BioFire respiratory panel (bioMerieux) was used for rapid testing and diagnostic services were extended from five days per week between eight to 17 hours to seven days per week between eight to 22 hours during the 2014-2015 respiratory season (Dec. 11, 2014 to April 5, 2015) as part of a new adequate and LEAN diagnostic policy at University Medical Center Groningen.

The researchers found that over the study period 641 tests were ordered in the emergency department and of those, 492 contained all needed data for analysis. The median turnaround time was 110 minutes at the medical microbiology department (from sample to answer), yielding a total median time to result of 165 minutes (from patient registration at the emergency department to final result and medical

decision). While only 15 percent of samples (n=51) tested positive for influenza, 24 percent (n=79) tested positive for coronavirus, rhinovirus, human metapneumovirus, RSV, PIV or influenza B. In the vast majority of positive cases (93 percent), results were available before admission for 330 patients, which, the researchers say, improved the bed management and in-hospital patient flow. Furthermore, timely negative results prevented 186 patients from going in isolation.

"While there is a specific therapeutic intervention available for outpatients who test positive for influenza viruses (oseltamivir), the implications of testing positive for a non-influenza virus are unclear."

— David Peaper, M.D., Ph.D.

Yet, in the outpatient settings, more targeted testing approaches may be more cost-effective, according to Yale researchers presenting at the conference.

Researchers evaluated measures associated with testing positive for influenza and non-influenza RV among outpatients at the West Haven Veterans Administration Hospital (Connecticut). The FilmArray Respiratory Panel (Biofire) was used from Dec. 15, 2014 to April 15, 2015 in 408 outpatients seen in the emergency department, off-site urgent care, and outpatient clinics. Differences in antibiotic and oseltamivir prescription rates were analyzed through medical records.

Among the 295 patients managed as outpatients, 105 tested positive for influenza, 109 tested positive for a non-influenza pathogen, and 81 tested negative. Those that tested positive for influenza viruses received fewer antibiotic prescriptions and more oseltamivir prescriptions than those who tested positive for another pathogen or those who tested negative.

"While there is a specific therapeutic intervention available for outpatients who test positive for influenza viruses (oseltamivir), the implications of testing positive for a non-influenza virus are unclear," writes co-author David Peaper, M.D., Ph.D., Yale-New Haven Hospital in New Haven, Conn. "There was no difference in antibiotic prescription rates among those who tested positive for a non-influenza pathogen compared to those who tested negative, suggesting that testing for influenza viruses alone may be sufficient and more cost-effective than multiplex pathogen testing for outpatients with respiratory tract infections."

Takeaway: Comprehensive panels for RVs may be more cost effective to use in certain hospital populations, like the emergency department and ICUs, compared to outpatient settings.

TB Diagnosis May Be Improved With New Three-Gene Panel

A three-gene set can robustly diagnose active tuberculosis (TB) from whole blood samples, according to a study published in the March issue of *The Lancet Respiratory Medicine*. The authors say that the three-gene set represents an improvement over current diagnostics because it is based on peripheral blood rather than sputum samples, it performed well in the diagnosis of latent tuberculosis versus culture-positive active tuberculosis in children, and HIV status did not change its diagnostic power.

"The multiple gene expression diagnostics that have been derived all suggest that host-response gene expression assays are likely to eventually play an important role in TB diagnostics," writes lead author Timothy Sweeney, M.D., from Stanford University.

TB remains a large, worldwide public health issue with nearly 10 million new infections per year. Yet, active pulmonary disease remains difficult to diagnose and treatment response is difficult to monitor. Traditional methods such as skin testing and interferon γ release assays are unable to distinguish between latent and active disease. Even newer technology has challenges (sputum-based, reduced accuracy in HIV-positive patients, and not applicable for treatment response monitoring). The World Health Organization (WHO) has called for new non-sputum diagnostics to meet the targets for TB prevention, care, and control.

The present study used samples of active pulmonary tuberculosis infection in whole blood from two public gene expression microarray repositories to derive a diagnostic gene set. Gene expression was assessed in heterogeneous patients from a wide variety of ages and 10 countries. Three datasets (n=1,023) were used for discovery and the gene set was then validated in 11 datasets (n=1,345 samples).

The researchers discovered the genes GBP5, DUSP3, and KLF2 are highly diagnostic for active tuberculosis. They were validated with a global area under the curve of 0.90 for distinguishing active TB from healthy controls; 0.88 for latent tuberculosis; and 0.84 for other diseases. When maximizing the sensitivity, the global threshold test characteristics across all validation datasets were: sensitivity 0.96 and specificity 0.56 for healthy controls versus active TB; sensitivity 0.95 and specificity 0.51 for latent versus active TB; and sensitivity 0.95 and specificity 0.47 for other diseases versus active TB. HIV infection status, bacterial drug resistance, or Bacillus Calmette–Guérin vaccination did not affect expression of the three-gene set. In an additional four cohorts, the TB score declined during treatment datasets (the tuberculosis score fell by 0.02 to 0.05 per week) in patients with active TB, indicating a potential utility of the test for quantitative monitoring of TB treatment response.

The authors say that the small size of the gene set will aid its ultimate clinical application, by reducing costs and complexity.

"The high parsimony and internal normalization of the three-gene set could allow for a point-of-care application, because it might be possible to assay three genes with solar-powered polymerase chain reaction instruments," writes Sweeney and colleagues. "Although the three-gene set does not meet all needs of the consensus target product profile (e.g., overall sensitivity greater than 0.95 can only be achieved with specificity in the range of 0.50), it is able to fulfill several of the requirements of the consensus target product profile."

Additionally, the WHO target product profile highlighted a strong need for a new diagnostic with excellent sensitivity that uses non-sputum samples; maintains an overall sensitivity of greater than 80 percent in patients with HIV co-infection; achieves a sensitivity higher than 66 percent in children with culture-positive TB; and is relatively simple to run.

Takeaway: Although the diagnostic and monitoring properties of this three-gene set need to be confirmed by prospective validation with a targeted assay, a three-gene test can improve TB diagnosis by differentiating active disease from latent disease.

WHO High Priority Target Product Profiles for TB

- A point-of-care, non-sputum-based test capable of detecting all forms of TB by identifying characteristic biomarkers or biosignatures
- A point-of-care, triage test, which should be a simple, low-cost test to identify those who need further testing
- A point-of-care, sputum-based test to replace smear microscopy for detecting pulmonary TB
- A rapid drug-susceptibility test, used at the microscopy-center level of the health care system, to select first-line regimen-based therapy

G2 INSIDER

Nonfasting Lipids OK for Initial Testing; Labs Urged to Use 'Desirable' Cut-Offs to Flag Results

Non-fasting lipid profiles are recommended for most patients, including for cardiovascular risk assessment, according to a joint statement published by the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine April 26 in the *European Heart Journal*. The panel recommendations are expected to improve patient compliance with lipid testing, while laboratory flagging of abnormal values, based on desirable concentration cut-points, rather than by reference intervals, is called for.

Traditionally, lipid profiles have been measured using samples collected after fasting for at least eight hours, which does not reflect the daily average plasma lipid and lipoprotein concentrations and associated risk of cardiovascular disease, since most people are in a non-fasting state for the majority of the day. Recently, though, "extensive" evidence show that changes in lipid and lipoprotein protein levels after a meal are only "modest" and not clinically significant for triglycerides, total cholesterol, LDL cholesterol or calculated remnant cholesterol, while concentrations of HDL cholesterol, apolipoprotein A1 and B, and lipoprotein(a) are not affected by fasting status.

"Numerous prospective cohorts have found significant associations for non-fasting lipids, lipoproteins, and apolipoproteins with cardiovascular disease risk, and several landmark clinical trials of statin therapy have used non-fasting lipids for trial entry criteria and for monitoring the efficacy of lipid-lowering therapy," the panel noted. "Collectively, these observations suggest that non-fasting blood sampling is highly effective, practical, and advantageous in assessing lipid-mediated cardiovascular disease risk and treatment responses."

The panel recommends that fasting is not required for routine plasma lipid profile or for cardiovascular risk assessment in most patients, including children, diabetic patients, and the elderly. They also suggest that for non-fasting samples, laboratory reports should flag abnormal concentrations as triglycerides ≥2 mmol/L, total cholesterol ≥5 mmol/L, LDL cholesterol ≥3 mmol/L, calculated remnant cholesterol ≥0.9 mmol/L, calculated non-HDL cholesterol ≥3.9 mmol/L, HDL cholesterol ≤1 mmol/L, apolipoprotein A1 ≤1.25 g/L, apolipoprotein B ≥1.0 g/L, and lipoprotein(a) ≥50 mg/dL (80th percentile).

However, the panel still recommends fasting when non-fasting plasma triglyceride concentration >5 mmol/L, a patient has known hypertriglyceridemia, is starting medications associated with severe hypertrigylceridemia, and following hypertriglyceridemic pancreatititis.

Additionally, in areas where fast food consumption is especially high, fasting may be recommended or patients can be warned to avoid high-fat food the day of the test. "Fasting is less critical for first-stage screening, but may be more important when trying to establish a phenotypic diagnosis of genetically determined dyslipidaemias," the panel writes.

Company References

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Tetracore 240-268-5400

U.S. Centers for Disease Control and Prevention
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U.S. Preventive Services Task Force
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