

Summer 2015



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14-3-3 η : A Joint-specific Biomarker in the Diagnosis of Rheumatoid Arthritis and Psoriatic Arthritis

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The prevalence of rheumatoid arthritis (RA) in the United States is 0.5% to 1%, affecting over 1.5 million adults.¹ Approximately 165,000 new cases are diagnosed annually.¹ If the disease is diagnosed early (ie, before significant joint erosion occurs), treatment can prevent irreversible joint damage. However, early diagnosis is difficult for several reasons: joint symptoms and signs are limited, physical findings to suggest synovitis may be absent, and laboratory test results may be seronegative in some patients. This article will briefly describe why new biomarkers are needed

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for RA, the blood tests that are currently available, and the clinical gap. A novel biomarker (14-3-3 η), its pathogenetic mechanisms, and the scientific basis for its use as a diagnostic test will also be described. Diagnostic applications include assessing risk for RA, diagnosing RA, determining joint damage prognosis, and monitoring RA therapy. Finally, the role of 14-3-3 η in patients with psoriasis will be discussed.

The Need for New Biomarkers

According to the European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) RA classification criteria, 40% of the total possible score for a classification of RA relies on blood test results.² However, many patients with RA have normal blood test results. In an observational study of 2370 patients in Finland and the US conducted between 1980 and 2004, the erythrocyte sedimentation rate (ESR) was normal in 45% to 47% of patients with RA. C-reactive protein (CRP) was normal in 44% to 58%, and rheumatoid factor (RF) was negative in 37% to 38%. All 3 tests were normal in 14% to 15%.³ Furthermore, the OMERACT (Outcome Measures in Rheumatology) group established BioDam (Biomarkers of Damage) to identify surrogates of radiographic progress to improve strength of prediction. They reported that current baseline variables explain only 32% of the variance in outcomes in RA patients over 5 years.⁴ Thus both of these studies demonstrate a need for better biomarkers.

Current Biomarkers

As shown above, current biomarkers are limited. ESR and CRP are markers of systemic inflammation and therefore not specific to joint inflammatory processes. RF is commonly elevated in chronic inflammation or chronic infection and thus has low specificity in early and established RA. Cyclic citrullinated peptide (CCP) has comparable sensitivity to that of RF but is highly specific for RA. Both markers are less sensitive in early

disease. Cytokines such as matrix metalloproteinase 3 (MMP3) may be generated anywhere in the body and are not RA specific.

Presymptomatic RA

Evolving models of RA describe a progression from genetic risk (affected first-degree relative, HLA-DR4 positivity) and environmental risk (smoking, gingivitis, *Porphyromonas gingivalis*) to preclinical autoimmunity (RF, CCP) to clinical disease.⁵ Individuals without clinical joint findings or histological synovial changes, but with RF and CCP positivity or positivity for 2 RF isotypes, are at risk of progressing to RA.⁶⁻⁷ These studies show that the presence of RF or CCP does not necessarily correlate with the presence of histological joint inflammation. So we cannot know when a seropositive, asymptomatic individual is actually developing joint inflammation. We cannot detect joint inflammation until overt symptoms of morning stiffness and findings of joint swelling, heat, tenderness, erythema, and loss of function appear. That is, we cannot identify at-risk individuals in the presymptomatic phase prior to development of joint inflammation. This precludes institution of proposed treatments to prevent progression to clinical disease.

14-3-3, a Family of Chaperonin Proteins

14-3-3 proteins are a family of ubiquitous shuttle proteins that move proteins intracellularly. They are involved in regulation of the cell cycle, intracellular trafficking/targeting, signal transduction, cytoskeletal structure, apoptosis, and transcription. The name 14-3-3 derives from their initial isolation as the 14th fraction on DEAE-cellulose column elution and localization in the 3-3 position on 2D starch gel electrophoresis. Structurally, these proteins function as dimers that form a cup-shaped structure with a large central 40 Å (4 nm)-wide deep groove containing 2 amphipathic grooves. Proteins with phosphorylated serine or threonine in motifs RSXpSXP and RX(Y/F)XpSXP bind

via salt bridges within the amphoteric grooves.⁸⁻⁹

14-3-3 proteins affect function in 1 of 3 ways: 1) *clamping function*: 14-3-3 interacts with a binding partner, promoting a conformational change in the target and altering its biological function; 2) *masking*: interaction between 14-3-3 and its binding partner blocks interactions between the binding partner and other intracellular proteins; 3) *scaffolding*: enables 2 proteins to come in close proximity to one another, allowing them to carry out their respective biological functions.¹⁰⁻¹¹ The 14-3-3 protein genes map to chromosome 22q12.3 and encode a 246-amino acid polypeptide chain with a predicted molecular weight of about 28 kilodaltons. These proteins are highly homologous across species. There are 7 known isoforms with 50% amino acid homology: β (beta), ϵ (epsilon), γ (gamma), τ (tau), ζ (zeta), σ (sigma, stratifin), and η (eta). Isoforms occur as homodimers and heterodimers, except mammalian σ which is only found as a homodimer.

14-3-3 η

The 14-3-3 η isoform comprises about 1% of brain proteins and is released from synovial fibroblasts in RA. How 14-3-3 η is released from synovial fibroblasts is not fully understood. It is thought to be released from exosomes because 14-3-3 proteins are key components of small lipid vesicles, and it is known that extracellular expression of 14-3-3 proteins from keratinocytes is exosomally mediated. Synovial fibroblasts likely release 14-3-3 η through activation by immune cells including dendritic cells, T-cells, B-cells, and macrophages.¹²

14-3-3 η is present in synovial fluid and serum in patients with RA; levels are consistently higher in the synovial fluid than in the serum in these patients.¹² Serum levels are at least 3 times higher in patients with RA than in healthy controls.¹³

14-3-3 η Induction of Metal

Metalloproteinase 9 (MMP-9) Production

14-3-3 η stimulation of macrophages leads to MMP-9 and tumor necrosis factor alpha (TNF α) protein release as determined through analysis of tissue culture supernatants. Extrinsic 14-3-3 η stimulates production of itself and TNF α , and TNF α stimulates production of itself and 14-3-3 η in a proinflammatory amplification loop. Other joint tissues may be involved in generating 14-3-3 η in the joint. TNF α stimulation of 14-3-3 η has been reported in ATDC5 chondrocytes.¹⁴

Considering the involvement of 14-3-3 η in promoting joint inflammation, it can be considered a mechanistic biomarker as defined by Robinson, since its functions include the regulation of intracellular communication networks that are involved in inflammatory processes relevant to RA.¹⁵

Measuring 14-3-3 η

14-3-3 η is measured using a sandwich enzyme-linked immunosorbent assay (ELISA) that employs 2 proprietary monoclonal antibodies specific to the η isoform. The protein is stable at room temperature and 4°C for 7 days and for at least 3 freeze-thaw cycles. Detection is not impacted by common interfering substances found in serum, nor is it impacted by RF. There are no significant changes with age (adults), gender, or ethnicity.¹⁶

14-3-3 η as a Diagnostic Marker for RA and Erosive Psoriatic Arthritis (ePsA).

Serum 14-3-3 η distinguishes patients with established RA from healthy individuals and those with other disorders (Figure 1).¹⁶ Using a serum 14-3-3 η cutoff of ≥ 0.19 ng/mL resulted in a sensitivity and specificity of 77% and 93%, respectively, and a corresponding likelihood ratio (LR) of 10.4 for established RA (Figure 2). Using the same cutoff, 64% of patients with early RA were positive for 14-3-3 η , with a corresponding

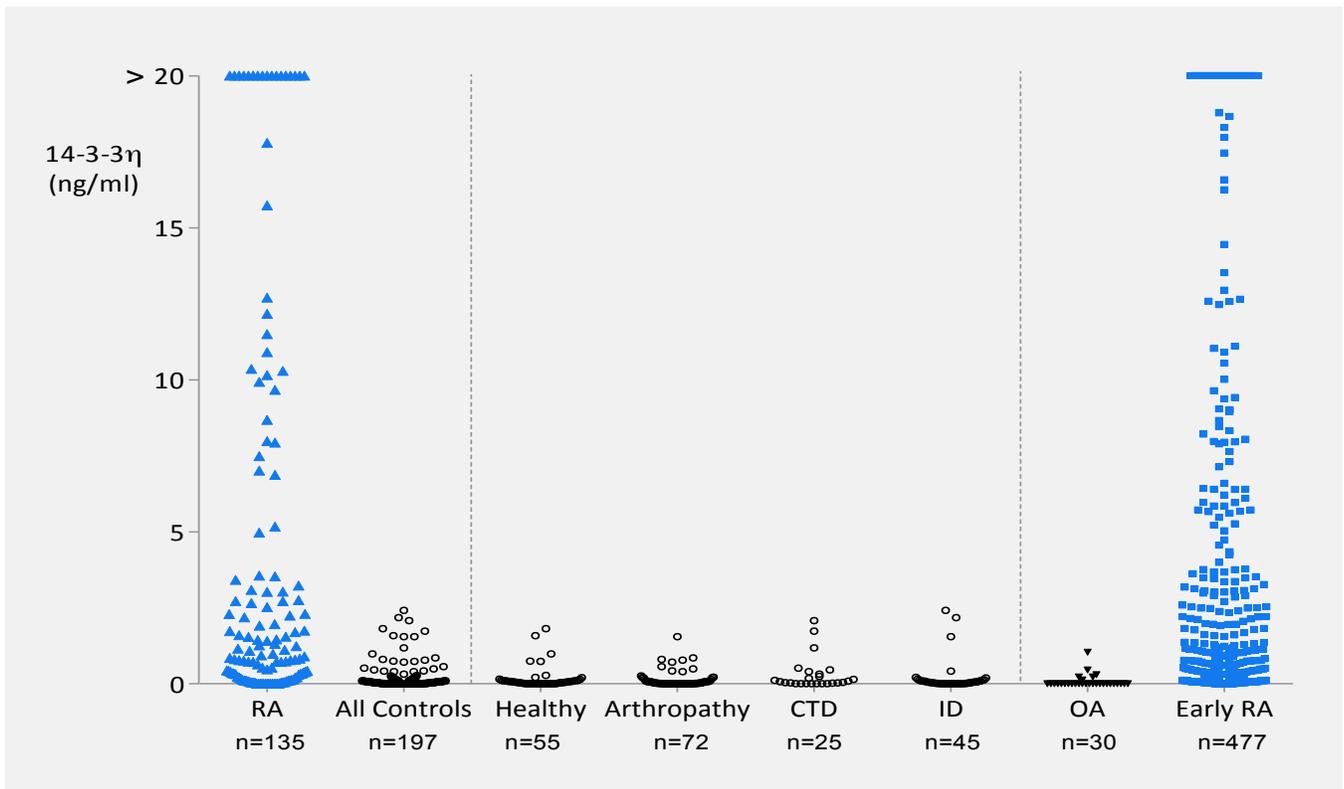


Figure 1: Serum 14-3-3 η expression in established and early rheumatoid arthritis (RA) versus controls. CTD, connective tissue disease; ID: inflammatory disease; OA: osteoarthritis. Maksymowych WP, Naides SJ, Bykerk V, et al. *J Rheumatol.* 2014;41:2104-2113. (Used with permission.)

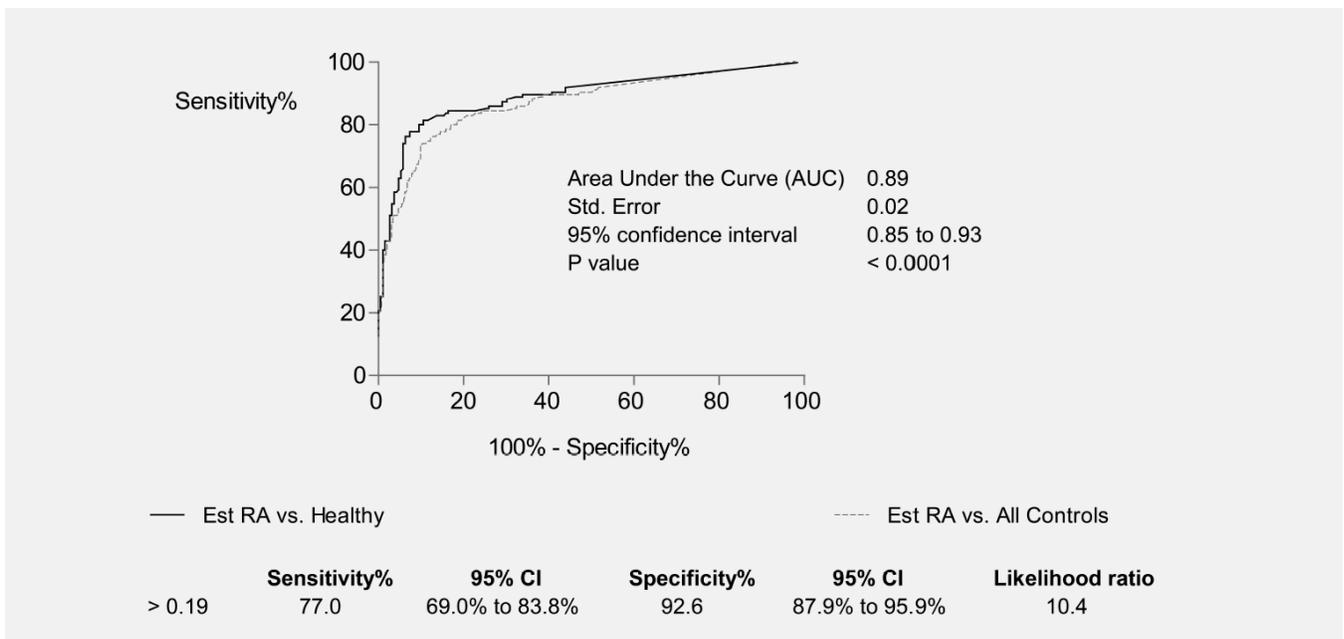


Figure 2: Corresponding receiver-operating characteristic curve for established RA versus controls. Maksymowych WP, Naides SJ, Bykerk V, et al. *J Rheumatol.* 2014;41:2104-2113. (Used by permission)

specificity of 93% (LR of 8.6). In comparison, 59% and 57% were positive for CCP antibody and RF, respectively, in early RA. Combined use of CCP antibody, RF, and 14-3-3 η resulted in a sensitivity of 78% for early RA. There was no correlation between 14-3-3 η and ESR, RF, CCP, or disease activity score (DAS). A positive 14-3-3 η result did correlate with significantly worse disease, however.¹⁶ Further, patients with ePsA had mild to moderately elevated 14-3-3 η , whereas patients with psoriasis without arthritis or psoriatic arthritis patients without erosions did not.¹⁶ Thus, 14-3-3 η is a useful diagnostic marker in RA and has utility in identifying psoriasis patients who have erosive joint disease.

14-3-3 η in Osteoarthritis (OA) Patients

In a study of 14-3-3 η specificity for RA, we examined physician-diagnosed OA patients in the U.S.-based Arthritis Internet Registry (AIR), a collaboration between the Arthritis Foundation, the National Data Bank for Rheumatic Diseases, and Quest Diagnostics. Clinical data were obtained by questionnaire and physician and/or medical record inquiry. Of 166 participants, 11 (6.6%; 95% CI, 3.7% to 11.5%) were positive for 14-3-3 η . Three of these were subsequently reclassified as having either RA or PsA based on RF, CCP, and 14-3-3 η or subsequent clinical presentation. After excluding these 3, eight of 163 OA participants were 14-3-3 η positive (4.9%; 95% CI 2.5%–9.4%). Thus, the specificity in OA patients for 14-3-3 η as a marker of inflammatory arthritis was 95.1% (95% CI, 90.6%–97.5%).¹⁷ These data suggest 14-3-3 η may be used to help identify RA or PsA in patients diagnosed with OA, either correcting a misdiagnosis or detecting concurrent inflammatory arthritis.

14-3-3 η in “Healthy” Donors

In a study of 134 healthy, community dwelling, ambulatory donors without a history of arthritis or

autoimmune disease, 6 (4%) had elevated 14-3-3 η levels. Of these 6, 1 had elevated 14-3-3 η of 9.22 ng/mL and elevated IgM RF (25 U/mL; RI <6 U/mL) and elevated IgG RF (13 U/mL; RI <6 U/mL), but normal RF IgA (RI <6 U/mL), consistent with increased risk of developing RA.^{16,18}

Serum 14-3-3 η Prediction of RA Risk

Patients presenting with arthralgia and an elevated baseline 14-3-3 η , but not a single swollen joint to meet 2010 EULAR/ACR classification criteria, had a 5.7 likelihood ratio of developing RA within 5 years.¹⁹ Patients with a positive 14-3-3 η have significantly higher clinical and serological measures of RA.¹⁶ Median 14-3-3 η levels are higher in patients with erosive disease.²⁰

Baseline 14-3-3 η Prediction of Joint Damage in Patients with Early RA

Early RA patients with elevated 14-3-3 η had worse Sharp radiographic joint damage scores at 5 years compared to those patients with normal baseline 14-3-3 η . The 14-3-3 η positive patients experience Sharp score deterioration beginning at 2 years compared to the 14-3-3 η negative group which began to show deterioration at 4 years. Thus, baseline 14-3-3 η predicts an aggressive joint damage trajectory.¹⁹

14-3-3 η as a Predictor of Response to Disease Modifying Antirheumatic Drugs (DMARDs) and Anti-TNFs

In those patients treated for 1 year with DMARDs, a decline in 14-3-3 η level from pretreatment baseline was associated with improvement in physician global assessment and disease activity score (DAS28).²¹ Perhaps of more clinical utility, in patients treated with TNF α inhibitors for only 15 weeks, a decline in 14-3-3 η level from pretreatment baseline was associated with improvement in DAS28, ESR, and CRP.²²

14-3-3 η , a Marker of Erosive Arthritis in Psoriasis

Patients with psoriasis have normal 14-3-3 η levels, as do psoriasis patients with nonerosive arthritis. However, patients with erosive psoriatic arthritis may have elevated 14-3-3 η . These elevations are usually not as high as those seen in RA. Hence, elevations in 14-3-3 η in followed psoriasis patients may herald onset of erosive disease.¹⁶

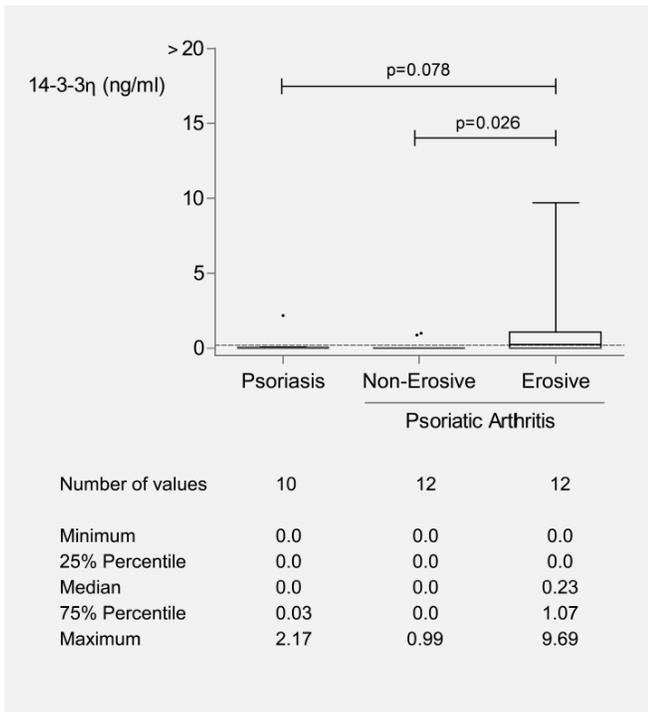


Figure 3: Expression in psoriasis and psoriatic arthritis. Maksymowych WP, Naides SJ, Bykerk V, et al. *J Rheumatol.* 2014;41:2104-2113. (Used by permission)

In summary, new biomarkers of RA are needed for earlier diagnosis, which can lead to better outcomes. New biomarkers are also needed to identify patients at high risk for an aggressive disease course, enabling optimal therapy. 14-3-3 η is a novel protein biomarker that is directly involved in disease pathophysiology, is present very early in disease, and informs progression risk and therapy response.

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Dr. Naides is a board certified internist with subspecialty certification in rheumatology. He received his bachelor's degree from Princeton University in biochemistry and attended the Hebrew University in Jerusalem. He received his M.D. from Hahnemann University. He trained in Internal Medicine at the University of Miami/Jackson Memorial Hospital and at Temple University. He completed fellowships in rheumatology at the University of California, San Diego and in immunology research at Harvard Medical School. Dr. Naides next joined the Medicine faculty, Division of Rheumatology, University of Iowa where he was an Assistant Professor then tenured Associate Professor and the founding Director of the interdisciplinary Helen C. Levitt Center for Viral Pathogenesis and Disease. He subsequently was Professor of Medicine, Microbiology & Immunology, and Pharmacology at The Pennsylvania State University/Milton S. Hershey Medical Center where he held the Hallowell Endowed Chair, was Chief of Rheumatology, and Co-Director of the Immunobiology Graduate Program. His extramurally supported basic and translational research has focused on viral pathogenesis and autoimmunity. He has served on NIH and foundation grant review study sections and continues to serve on national professional society committees. He is the author of more than 100 peer-reviewed papers, book chapters, and reviews. Dr. Naides joined Quest Diagnostics Nichols Institute in 2006 as Medical Director, Immunology R&D. He also serves as Interim Scientific Director for Immunology and Medical Director for Immunology in the Infectious Disease/Immunology Franchise in Quest Diagnostics.

A Novel Method to Measure Intact Insulin by Mass Spectrometry

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Insulin produced by the pancreatic beta cells is a critical regulator of intermediary metabolism. Adequate insulin levels are necessary to control blood glucose levels, to regulate fatty acid metabolism, and to restrain glucose production by the liver.^{1,2} Insulin deficiency is most often mediated by autoimmune destruction of the beta cell mass, as encountered in type 1 diabetes mellitus. Elevated levels of insulin can be caused by insulin-producing tumors and several other conditions, but insulin resistance is the most frequent.

Insulin resistance is characterized by an impaired biologic response, creating a *relative* lack of insulin. Insulin resistance is associated with a spectrum of conditions, including obesity, metabolic syndrome, and type 2 diabetes mellitus. In its earliest stages, insulin resistance is characterized by normal metabolism but elevated insulin. As the insulin-resistant state progresses, increased levels of insulin are required to regulate gluconeogenesis and fatty acid metabolism. Eventually, the level of resistance exceeds the insulin secretory capacity of the beta cells, and hyperglycemia ensues.^{2,3,4}

If diagnosed early, insulin-resistant states can often be reversed by exercise or medication.⁵ This, combined with the established role of insulin resistance as a precursor to full-blown diabetes mellitus, suggests that tools to assess insulin resistance states early and accurately would be of considerable value. Several studies, mostly retrospective, have also suggested

the utility of fasting insulin measurement in predicting future diabetes mellitus.⁶⁻¹⁰ However, although insulin measurement is widely studied in the research setting, it is generally not part of routine clinical practice.

One obstacle to incorporating insulin measurement into clinical practice is lack of assay standardization. Insulin is most often measured using immune-based assays, and wide interassay discrepancies in insulin values have been reported.¹¹ Assay specificity, calibration procedures, specimen type, assay performance, and conversion factors may all contribute to inter-assay variation in immune-based insulin assays.¹¹ These issues have restricted the capacity to compare measurements between different studies and to clearly define the role of insulin levels in diagnosis and prediction of insulin-resistant states.^{12,13}

To address these concerns, Quest Diagnostics has developed the first commercially available liquid chromatography-tandem mass spectrometry (LC/MS/MS) assay to measure intact insulin. Mass spectrometry-based assays have the advantage of differentiating possible cross-reactive components by molecular weight. This assay has been carefully constructed to permit the ready comparison to accepted standard materials and to allow conversion to SI units. This high-throughput method is almost entirely automated and can distinguish native insulin from all exogenous analogs. In addition to being highly specific, the assay has a sensitivity equivalent to immunochemical levels of detection.

Assay Methods

Enrichment of insulin from patient sera (150 μ L) is achieved by using monoclonal antibodies immobilized on magnetic beads. Samples are processed on a robotic liquid handler. Peptides are separated by reversed-phase high-performance liquid chromatography using an Aria TLX-4 System with

aqueous formic acid to acetonitrile gradients. A triple quadrupole mass spectrometer is used for detection and quantitation.

The assay has an analytical measurement range of 3 to 320 $\mu\text{IU/mL}$ (18 to 1,920 pmol/L) for insulin, with intra- and inter-day assay variation $<11\%$. Of the insulin analogues commonly prescribed to treat diabetes, only recombinant human insulin (insulin lispro) causes significant interference for determination of endogenous insulin.

We have standardized the assay by calibration to the WHO insulin reference material 83/500. However, we ensured that our standardization is SI traceable by performing quantitative amino acid analysis of all reference materials to establish peptide content independent of manufacturers. We obtained excellent agreement with the WHO-traceable Beckman Assess ICMA (Brea, CA) assay for most patient samples, and also independently confirmed accuracy using USP insulin in our controls for which peptide content was established by quantitative amino acid analysis (AAA).

Summary

The Quest Diagnostics assay for the measurement of insulin solves a long-standing problem in endocrinology. This method permits the precise and reproducible quantitation of insulin in an assay that is directly related to the mass of the peptide. The direct referencing to available international standards will permit consistent measurement and facilitate comparison of assay results performed in different studies and at different times.

Although not as readily identified as a critical detriment, C-peptide measurements have also been poorly standardized.¹⁴ The assay described herein also can be readily adapted as a multiplexed method for the simultaneous determination of intact insulin and C-peptide by immunocapture LC-MS/MS techniques.

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Michael J. McPhaul, M.D., Medical Director, Endocrine Division

Dr. McPhaul joined Quest Diagnostics in June 2012. Prior to his recruitment to Quest, Dr. McPhaul was Professor of Internal Medicine in the Division of Endocrinology and Metabolism at UT Southwestern Medical Center. During his 25 year tenure at Southwestern, his research career was clinical and translational in nature, leading to an international reputation in the fields of androgen action, diseases of the pituitary, and clinical research. He has published extensively, including 95 peer-reviewed manuscripts and 22 reviews and book chapters.

Since joining Quest as Medical Director for Endocrinology and Metabolism, Dr. McPhaul has focused primarily on: 1) the development of guideline supported panels to assist in the comprehensive care of patients with prediabetes, the metabolic syndrome, and diabetes mellitus, and 2) the development of new diagnostic tests to permit the early identification of patients with diabetic complications.

Dr. McPhaul is an active member of a number of professional organizations, including AACE, the Endocrine Society, the American Diabetes Association, and the American Federation for Medical Research. He serves as the Editor-in-Chief for two journals published by the AFMR, the Journal of Investigative Medicine and the Journal of Investigative Medicine High Impact Case Reports.

Latest News

Quest Diagnostics Announces LeukoVantage™—Advancing Precision Medicine for Hematologic Malignancies **Genomic service detects 30 gene mutations to aid in the diagnosis, prognosis, monitoring, and treatment selection for several myeloid neoplasms**

Quest announces the availability of LeukoVantage™, an evidence-based genomic test service that aids in the diagnosis, prognosis, monitoring, and selection of treatment for myeloid neoplasms, a group of hematologic malignancies that includes acute myeloid leukemia (AML), the most common form of adult acute leukemia, as well as myelodysplastic syndrome (MDS) and myeloproliferative neoplasms (MPNs).

“LeukoVantage is an outgrowth of Quest’s commitment to advancing oncology care based on actionable molecular insights,” said Frederick K. Racke, M.D., Medical Director, Hematology Oncology, Quest Diagnostics. “There is growing evidence that several genetic alterations involved in myeloid neoplasms not only provide significant diagnostic and prognostic value, but may also help guide treatment decisions. LeukoVantage builds from the latest science and Quest’s deep experience in genomics and hematology to deliver insights that can help the physician potentially diagnose the patient more quickly and reliably and establish an appropriate treatment plan.”

A wide range of gene mutations are associated with myeloid neoplasms, which occur when the bone marrow makes too many blood cells or platelets. Next-generation sequencing technologies can simultaneously test for mutations in multiple genes associated with myeloid neoplasms to diagnose,

subclassify, monitor, and predict the course of disease. LeukoVantage is a lab-developed test that uses next-generation sequencing, polymerase chain reaction (PCR), and other techniques to interrogate DNA from leukocytes (white blood cells) in blood or bone marrow specimens for the presence of mutations in 30 genes most frequently associated with myeloid neoplasms.

For MDS, LeukoVantage may detect certain molecular markers to help identify early stages of the disease, complementing conventional diagnostic techniques. MDS is typically diagnosed through a series of tests that include complete blood count (CBC), morphology, and cytogenetic (chromosomal) analysis. For MPN, LeukoVantage may identify molecular markers that can aid the selection of certain targeted therapies, such as JAK2 inhibitors, and establish a prognosis.

LeukoVantage has also been shown to detect gene mutations in greater than 95% of newly diagnosed cases of AML. Physicians may use this information to aid in disease diagnosis and subclassification as well as to establish a treatment plan with induction chemotherapy. In addition, LeukoVantage may aid in establishing a prognosis and in monitoring for minimal residual disease and recurring disease. In the case of both AML and MDS, LeukoVantage may also provide information for selecting epigenetic modifying drugs, which may be less toxic than inpatient chemotherapies.

In addition to LeukoVantage, Quest Diagnostics provides an industry-leading menu of hematologic test services, including morphology, flow cytometry, cytogenetics, FISH, and molecular services for blood and bone marrow specimens and other tissues and body fluids as necessary. Because patients with hematological cancers often require multiple services, clinicians may prefer a single provider that can coordinate, perform, and interpret all necessary

testing services as well as split bone, blood, and other samples to simplify test ordering and reporting.

“LeukoVantage is an innovative and clinically useful lab test—but that is only part of its value. Quest Diagnostics provides a broad suite of accompanying services for diagnosing and managing patients with myeloid neoplasms. Those larger capabilities can be most useful for these and other diseases in which continuous monitoring, supported by lab tests, is vital to promote quality patient care,” said Frederic Waldman M.D., Ph.D., Medical Director, Cancer Diagnostics, Quest Diagnostics.

LeukoVantage extends Quest’s growing menu of genomic oncology services. In June 2014, Quest Diagnostics launched OncoVantage™ in collaboration with Memorial Sloan Kettering Cancer Center, as a new molecular test for selecting chemotherapy based on somatic mutations of solid tumors, such as colon and breast cancer. Quest Diagnostics also provides genetic (germline) cancer services for assessing inherited risk of cancers, including BRCAVantage® for breast and ovarian cancers, and genetic testing for Lynch syndrome, which can cause colorectal and endometrial cancers. LeukoVantage builds on this expertise as well as precursor molecular panel sequencing services for MDS and MPN, which were among the first available to clinicians when introduced in the United States in 2013.

Quest Diagnostics to Enhance Quality of Noninvasive Prenatal Screening with QNatal Advanced™

New prenatal screening service provides actionable information for women with high-risk pregnancies

Quest announces the launch of QNatal Advanced, a noninvasive prenatal screening service for detecting chromosomal abnormalities in high-risk pregnancies.

QNatal Advanced analyzes cell-free fetal DNA in circulating maternal blood to screen for common and rare chromosomal abnormalities, including those associated with trisomy 21 (Down syndrome), trisomy 18 (Edwards syndrome), trisomy 13 (Patau syndrome) and monosomy X (Turner syndrome), as well as fetal sex aneuploidies and select copy number variants. The screening test analyzes more chromosomal regions than most other noninvasive prenatal tests, and can be used as early as the 10th week of gestation.

With results of a QNatal Advanced screen, a woman and her physician may be better positioned to assess the appropriateness of invasive diagnostic testing such as amniocentesis, which carries a slight risk of miscarriage.

“With QNatal Advanced, Quest Diagnostics is delivering on its commitment to provide clinically important innovations aligned with guideline-based care as part of a full range of capabilities that support a woman through her pregnancy,” said Charles (‘Buck’) Strom, MD, PhD, Vice President, Genomics and Genetics, Quest Diagnostics.

‘Noninvasive Prenatal Screening’— the Preferred Term

Prenatal diagnostic tests are not intended to provide diagnostic information under medical guidelines. To reduce the potential for confusion, Quest Diagnostics has adopted the phrase ‘noninvasive prenatal screening’ to describe QNatal Advanced in place of the common industry term ‘noninvasive prenatal test’ which may inappropriately connote a diagnostic test.

In addition, the company is only promoting QNatal Advanced as a screening service for women defined as high-risk under medical guidelines. These include

women 35 years of age at time of delivery and older or women with a personal and family history of an affected pregnancy, among other factors. According to guidelines, women of advanced maternal age may also benefit from noninvasive prenatal screening following an abnormal ultrasound or abnormal maternal serum screening test.

“As a leader in women’s health, Quest Diagnostics has a responsibility to ensure our patients and physicians understand both the advantages and limitations of the services we provide. Cell-free DNA tests are for screening only, and never diagnostic. The phrase ‘noninvasive prenatal screening’ coined by Quest Diagnostics reflects the seriousness with which we take our responsibility to ensure women have access to health insights that are appropriate, actionable, and of the highest quality,” said Douglas S. Rabin, MD, Medical Director, Women’s Health, Quest Diagnostics.

“If compelling new science emerges suggesting that these technologies can be used diagnostically, or that the benefits of screening average-risk women outweigh the risks, we will certainly reconsider our position. For now, we believe our approach is the right one to help women make the best possible decisions,” Dr. Rabin added.

Noninvasive Prenatal Screening—New and Improved

QNatal Advanced is a lab-developed test developed and validated by Quest Diagnostics using massively parallel shotgun sequencing (MPSS), a type of next-generation sequencing technology, with GC correction. Results of Quest’s validation study found QNatal Advanced provides excellent sensitivity and specificity, as well as low nonreportable results. Quest intends to present the findings at a scientific conference in the coming months.

QNatal Advanced is based, in part, on intellectual property licensed by Quest Diagnostics from Sequenom as part of an agreement announced in June 2014. Unlike Sequenom’s MaterniT21, QNatal Advanced provides the option to opt out of receiving information on gender and microdeletions, which can provide complex and limited actionable information, and facilitates direct ordering and reporting of results via Quest’s Care360 connectivity solution. It also reports results as Yes/No for the presence of relevant trisomies and other abnormalities, as compared to other industry reports that score findings on a likelihood scale.

“We’ve taken the best of the Sequenom technology and added to it our own expertise and innovation, based on our deep experience in genomics and women’s health,” Dr. Strom said. “The result is an offering that, while medically equivalent, may have greater potential to provide clear, actionable information to help each patient understand her personal risk.”

Quest Diagnostics offers a complete menu of diagnostic services to support healthy pregnancies. The company is believed to be one of the only lab providers to offer confirmatory CVS or amniocentesis following a noninvasive prenatal result. The company’s larger portfolio of services also include carrier screening for inherited genetic disorders such as cystic fibrosis and Jewish genetic screening such as Tay-Sachs disease, high-resolution chromosomal microarray analysis for unexplained developmental delay/intellectual disability in infants and toddlers, and physician access to genetic counselors and other medical experts for counsel in test selection and results interpretation.

Quest study shows how our new cancer test can help doctors with treatment recommendations

Many cancer therapies available today work by targeting certain gene mutations identified by laboratory testing. A new study by Quest shows that simultaneous testing for 34 genes may detect more mutations that doctors can use for treatment selection. The test, called OncoVantage™, uses next-generation sequencing to identify many gene mutations on a single patient specimen. Results are interpreted using clinical relevance information provided by Memorial Sloan Kettering Cancer Center.

The test may help improve the ability of community oncologists to identify people who will respond well to therapies for breast, colon, lung, and other solid tumor cancers.

The study was presented at ASCO (American Society of Clinical Oncology) 2015, the organization's annual meeting in Chicago. The meeting brings together 30,000 oncology professionals from around the world for educational sessions and research presentations.

"This next-generation sequencing (NGS) assay frequently detected actionable mutations not identified by routine single-gene tests," said Frederic Waldman, M.D., Ph.D., Medical Director, Cancer Diagnostics.

"These findings suggest that broader mutation profiling of solid tumors, as with the 34-gene NGS mutation panel, could provide additional information for treatment selection in a substantial proportion of cases."

Authors of the study included: Kevin Qu, Heather Sanders, Joseph Catanese, Andrew Grupe, Hai-Rong Li, Davis Ross, James Prentice, Lin Ma, Beryl Crossley,

Cindy Barlan, Xi Zhang, Jay Wohlgemuth, Anthony Sferruzza, John Sninsky, Feras Hantash and Dr. Waldman.

HPV-only Screening Misses More Cervical Cancers in Women 30 and Older Than Pap-only or Pap-HPV Co-testing Per a National Study

As many as 19% of women received false negatives for cervical cancer when only HPV was tested, according to a Quest Diagnostics Health Trends™ study published in *Cancer Cytopathology*. The study, which included 256,648 women, is one of the largest studies of the different cervical cancer screening tests.

HPV-only screening is less likely to accurately detect cervical pre-cancer and cancer than testing that includes a Pap test in women 30-65 years of age, according to a new study published online in *Cancer Cytopathology*, a peer reviewed journal of the American Cancer Society. The study, by researchers at Quest Diagnostics (NYSE: DGX) and the University of Pittsburgh Medical Center (UPMC), reinforces medical guidelines recommending women in this age group be screened with both Pap and HPV tests to rule out cancer risk. These guidelines, however, have come into question following FDA approval of an HPV-only screening test last year.

The study is believed to be one of the largest to examine the effectiveness of HPV and Pap screening for cervical pre-cancer and existing cervical cancer. It is based on approximately 8.6 million women 30-65 years of age who received concurrently performed Pap and HPV tests (co-testing) at Quest Diagnostics laboratories in the United States. Of these, 256,648 women also received a biopsy to detect cancer, and 526 had confirmed cases of cervical cancer.

According to the analysis, 18.6% of women with confirmed cervical cancer received a negative test for human papillomavirus (HPV), compared to 12.2% that had a negative Pap test and 5.5% that had a negative co-test result. Of 169 women with confirmed cervical adenocarcinoma, the most difficult form of cervical cancer for which to screen, 26.6% received a negative HPV test; this is compared to 20.7% that had a negative Pap test and 8.3% that had a negative co-test result.

“Our study arrives at a crucial moment in the evolution in cervical cancer screening for millions of women in the United States,” said co-author R. Marshall Austin, M.D., Ph.D., Professor of Pathology at Magee-Womens Hospital of UPMC. “Our large-scale, real-world patient data provides convincing evidence that HPV-only testing would tragically miss many cervical cancers that would be detected if co-testing were employed. In fact, our study showed that Pap reliably detected more cervical cancers than HPV-only, an incredibly important finding in light of the current debate about the use of HPV-only as a primary cervical cancer screen. Cervical cancer screening has been one of the great success stories in cancer prevention, and we hope that the medical community takes these findings seriously as it considers the best screening approach to promote favorable health outcomes for women.”

Medical guidelines have recommended that women 30-65 years of age be screened periodically for cervical cancer with HPV-Pap co-testing. HPV tests identify the presence of the virus that causes most cervical cancers, while Pap tests identify cellular abnormalities in the cervix caused by HPV infection that could indicate the presence of cancer or precancerous cellular changes. Cervical cancer, particularly when detected in early stages, can typically be treated with a variety of measures.

In April 2014, the FDA approved an indication for the cobas® HPV test to be used alone as a primary cervical cancer screen in women 25 years of age and older. The FDA approval was based on a clinical trial that compared HPV testing to Pap testing and involved eight confirmed cervical cancer cases. While the American Congress of Obstetricians & Gynecologists (ACOG) continues to recommend co-testing in women 30-65 years of age, the Society of Gynecologic Oncology and the American Society for Colposcopy and Cervical Pathology issued interim guidance in January 2015 citing HPV-only testing as a viable alternative to Pap-HPV co-testing or Pap alone. “Cervical cancer was the leading cancer killer of women in the United States before the introduction of the Pap test. Our latest Quest Diagnostics Health Trends study involving one of the largest populations of co-tested women shows that the Pap test should continue to play a front-line role in the battle against cervical cancer,” said Douglas S. Rabin, M.D., Medical Director, Women’s Health, Quest Diagnostics.

Large Study Finds Unreliable HPV Test Results in Women with Cervical Cancer

The investigators analyzed results of HPV tests and Pap tests in samples from women 30-65 years of age for differences in rates of positive and negative tests results; the women were either co-tested, tested only by Pap, or tested only by HPV. Of the 256,648 women whose de-identified test results were analyzed for the study, 1.6%, or 4,090, had intraepithelial neoplasia stage 3 (CIN3), a premalignant condition, or more severe biopsy results. Proactive management of CIN3 is recommended to minimize the risk of cancer.

Key findings:

- **HPV alone missed more confirmed cancers than Pap alone.** Among 526 women with a diagnosis of cervical cancer, 18.6% were HPV negative compared

to 12.2% who were Pap-test negative and 5.5% who were co-test negative. This is an approximately three-fold improvement in the cancer detection rate of co-testing compared to that of HPV alone. In addition, 26.6% of women with cervical adenocarcinomas tested HPV negative.

- **Co-testing identified more cases of CIN3 and more severe results.** Co-testing detected 98.8% of women with CIN3 or more severe cervical biopsy results, compared to 94.0% for HPV-only testing and 91.3% for Pap-only testing. Among women who had an abnormal Pap test result, a negative HPV result, and a CIN3 or more severe cervical biopsy result, 35.4% had cervical cancer.
- **Older age associated with HPV-negative test results in women with cervical cancer.** The average age of women in the study who had a Pap test was 45.8 years of age. Women with HPV-negative cervical cancer were 52-53 years of age on average compared to 43-44 years of age on average for all HPV-negative patients studied.

The study, “Comparison of cervical cancer screening results among 256,648 women in multiple clinical practices,” is available at <http://onlinelibrary.wiley.com/doi/10.1002/cncv.21544/full>

“This study highlights that up to 19%, or about 2,400, of the approximately 12,400 women diagnosed with cervical cancer each year could be falsely reassured of a negative screening result were they screened by HPV-only testing. As early detection and treatment of cervical cancer are critical to a favorable outcome, it is important that the best and most sensitive diagnostic tools for cancer detection be identified and made available to all women. Our data support co-testing in women 30–65 years of age as the most effective screening test for cervical cancer detection,” Dr.

Rabin said.

According to the American Cancer Society, nearly 12,400 women are diagnosed with cervical cancer annually, and more than 4,000 will die of the disease. The incidence of cervical cancer deaths has declined dramatically in the United States “due to early detection as a result of screening with the Pap test.” More than half of new cervical cancer cases occur among women who have never or rarely been screened, according to the Centers for Disease Control and Prevention.

The present study’s strengths are its size, national scope, and use of unselected patient results rather than a control study as well as the use of quality laboratory methods. The HPV tests in the study may not share the precise performance characteristics as the cobas® HPV test and are not intended to be used as HPV-only screens. The study was performed in compliance with applicable privacy regulations and the company’s strict privacy policies and was determined to be exempt from Western Institutional Review Board.



Quest scientist plays role in developing steroid analysis guidelines

A Quest scientist, Nigel Clarke, Ph.D., played a critical role in developing guidelines for analyzing steroids on mass spectrometry. The guidelines, entitled “Clinical and Laboratory Standards Institute document C57 – Mass Spectrometry for Androgen and Estrogen Measurements in Serum,” are basically a set of rules for anyone planning to analyze steroids on mass spectrometry (MS).

“It has been a real honor to work on guidance documents for the Clinical and Laboratory Standards Institute (CLSI) with a group of key opinion leaders over the last few years,” said Dr. Clarke, who is Senior Science Director, Applied Technology, Mass Spectrometry and Immunology, and based in San Juan Capistrano, California. “These global guidelines are around the use of clinical MS and the first, just published, is guidance for MS measurement of androgens and estrogens.”

According to the CLSI, a not-for-profit organization devoted to fostering excellence in laboratory medicine, the primary objectives of the guidelines are to provide guidance and establish uniform practices necessary for producing quality data for quantitation of androgens and estrogens. The guideline provides details specific to androgen and estrogen measurement procedures with respect to preexamination (preanalytical) considerations, MS technologies, measurement procedure and run validation, as well as postexamination (postanalytical) considerations.

Quintiles and Quest Diagnostics to form joint venture to provide global clinical trials laboratory services

Quintiles and Quest Diagnostics have announced a definitive agreement to form a global clinical trials laboratory services business. By combining the clinical trials laboratory operations of two industry leaders, the new entity will provide customers with the depth of capabilities and end-to-end clinical trial laboratory services required to succeed in the increasingly complex biopharmaceutical industry. The new entity will offer a compelling value proposition to solidify its position as the second-largest central laboratory services company in the world. The transaction, which is subject to standard and customary closing

conditions, is anticipated to close in the third quarter of 2015.

The joint venture would have generated approximately \$575 million in revenues in 2014, and will offer a superior breadth of services and an industry-leading test menu to its customers across all segments of the biopharmaceutical industry—a group that includes all of the top-20 largest biopharmaceutical companies. Upon closing of the transaction, Quintiles will own 60% and Quest Diagnostics will own 40% of the new joint venture.

Quintiles’ scale, clinical trial expertise, and diverse therapeutic experience coupled with Quest Diagnostics’ operational, scientific, and quality excellence, supply chain network, and informatics promise greater innovation, quality, and value for biopharmaceutical customers. In addition, customers will benefit from a globally scaled and agile business model that is singularly focused on delivering top-quality laboratory services informed by deep medical and clinical knowledge.

Clinical, scientific, and statistical excellence is becoming increasingly important for successful laboratory work as part of the regulatory approval process. To meet these needs, the entity will draw upon the deep scientific, medical, and data expertise of Quintiles and Quest Diagnostics, which together have a team of approximately 3,500 medical doctors, Ph.D.s and biostatisticians as well as cutting-edge experience, expertise, and capabilities in the areas of genomics and precision medicine.

The new entity also will leverage a number of additional resources from the broader Quintiles and Quest Diagnostics organizations, including the award-winning Quintiles Infosario® technology platform and

Quest Diagnostics' robust data analytics capabilities. Both companies are bioinformatics leaders in their respective fields—positions that have been built upon and honed from diverse experience and data access. Quest Diagnostics draws from 20 billion test results, and Quintiles' assets include electronic health records representing more than 60 million patient lives and a network of 250,000 clinical investigators.

“This joint venture builds upon the best of Quintiles' and Quest Diagnostics' strengths, creating top-quality services for customers,” said Tom Pike, CEO, Quintiles. “It will join together the scale, expertise and end-to-end capabilities of the broader Quintiles and Quest Diagnostics organizations with a laser-like focus on providing world-class laboratory services that will help improve customers' probability of success. Quintiles and Quest Diagnostics are already industry leaders with complementary offerings, capabilities, and visions to improve patient outcomes and human health. We're excited about the opportunities ahead of us and the benefits we can provide customers.”

The joint venture will be led by an experienced global management team appointed from Quintiles and Quest Diagnostics. Built upon the strong histories and foundations of quality delivery of its parent organizations, the new entity will provide customers with a variety of advanced testing capabilities and services. These will enable end-to-end

companion diagnostic development with regulatory, reimbursement, and commercial strategies, to help biopharmaceutical customers' precision medicine efforts. It also will help enhance quality and efficiency by leveraging Quest Diagnostics' supply chain that serves approximately half of the physicians and hospitals in the U.S. and includes 2,200 service centers, 3,000 couriers, and connectivity solutions used by 250,000 healthcare providers.

“We're thrilled to partner with the world's leading biopharmaceutical services provider to help customers succeed with their clinical trials and support the pursuit of precision medicine,” said Steve Rusckowski, President and CEO. “For Quest Diagnostics, the joint venture model will enable us to generate growth and value from our clinical trials assets while simultaneously strengthening our focus on our core diagnostic information services business.”

Beyond the immediate opportunity in laboratory services support for clinical trials, Quintiles and Quest Diagnostics expect to benefit from opportunities to collaborate in other areas. These include new ways to enhance patient recruiting and retention for clinical trials; speed the validation, development, and commercialization of companion diagnostics; enhance support of real-world late phase studies; and develop new population-health analytics and tools.

New Assays at Quest Diagnostics

New challenges in medical sciences often require new solutions. Here we show some of our new methods that have been recently introduced.

Test Name	Description
LeukoVantage: Advancing precision medicine for hematologic malignancies.	A wide range of gene mutations are associated with myeloid neoplasms, which occur when the bone marrow makes too many blood cells or platelets. Next-generation sequencing technologies can simultaneously test for mutations in multiple genes associated with myeloid neoplasms. Test results can be used to diagnose, subclassify, monitor, and predict the course of disease. LeukoVantage is a lab-developed test that uses next-generation sequencing, polymerase chain reaction (PCR), and other techniques to interrogate DNA from leukocytes (white blood cells) in blood or bone marrow specimens for the presence of mutations in 30 genes most frequently associated with myeloid neoplasms.
FDA clears the Simplexa™ Group A Strep Direct Test from Focus Diagnostics, a Quest Diagnostics business.	Focus Diagnostics, the clinical-laboratory products business of Quest Diagnostics, announced the FDA 510(k) clearance and CLIA moderate-complexity categorization for its Simplexa Group A Strep Direct Kit. Simplexa Group A Strep Direct is a real-time polymerase chain reaction assay for the detection of Group A Streptococcus bacteria directly from throat swabs.
Quest Diagnostics to enhance diagnosis of pediatric neurological disorders with exclusive whole exome sequencing from Personalis.	Quest Diagnostics announced the availability of Neurome™, a whole exome sequencing service designed to aid the diagnosis of rare neurological disorders in pediatric populations. Personalis, Inc., a leading genomics-based diagnostic laboratory, developed and performs the lab-developed test exclusively for Quest Diagnostics. Neurome is the first whole exome sequencing service available through Quest Diagnostics and its Athena Diagnostics neurology diagnostics business.
Quest Diagnostics and Inserm launch multinational BRCA gene datashare initiative	Quest Diagnostics and Inserm, the French National Institute of Health and Medical Research institution, recently launched BRCA Share™, a novel datashare initiative they co-founded to provide scientists and laboratory organizations around the world with open access to <i>BRCA1</i> and <i>BRCA2</i> genetic data. The program's goal is to accelerate research on <i>BRCA</i> gene mutations, particularly variants of uncertain significance, to improve the ability of clinical laboratory diagnostics to predict which individuals are at risk of developing hereditary breast and ovarian cancers.

Test Name	Description
Pap with Age-based Screening Protocols	<p>These new test codes are designed to help clinicians order age-appropriate testing for cervical cancer screening and STIs. Based on the patient's age and on ACOG Practice Guidelines for cervical cancer screening and <i>Chlamydia trachomatis</i> (CT) and <i>Neisseria gonorrhoeae</i> (NG) screening, testing in addition to the Pap will be processed as follows:</p> <ul style="list-style-type: none"> • Patients aged 21-25: Patients will receive image-guided Pap and, when the result is ASC-US, a high-risk HPV mRNA test will be performed. Additionally, testing for CT/NG will be performed. • Patients aged 26-29: Patients will receive image-guided Pap and, when the result is ASC-US, a high-risk HPV mRNA test will be performed. • Patients aged 30-65: Patients will receive image-guided Pap and a high-risk HPV mRNA test. When the Pap result is negative but the HPV mRNA screen is positive, genotyping for HPV mRNA 16, 18/45 will be performed. • Patients falling outside of the above age ranges, or without a date of birth, will receive an image-guided Pap only. <p>Codes may include ThinPrep® or SurePath™ image-guided Paps, HPV mRNA, CT/NG.</p>
<i>Helicobacter pylori</i> Culture with Reflex to Susceptibility	<p><i>Helicobacter pylori</i> has been associated with peptic ulcer disease and cancers of the gastrointestinal tract. In 1994, the U.S. National Institute of Health Consensus Development Conference concluded that <i>H. pylori</i> infection represents the major cause of peptic ulcer disease. Antimicrobial treatment is recommended for patients with the confirmed disease.</p>
HPV mRNA E6/E7, Rectal with Reflex to Genotypes 16, 18/45	<p>This transcription-mediated amplification (TMA) HPV mRNA E6/E7 test is used in conjunction with cytology to evaluate anal dysplasia.</p>
HPV Genotypes 16, 18/45, Anal-Rectal	<p>This transcription-mediated amplification (TMA) assay is used in combination with anal and rectal cytology to assess the presence or absence of high-risk HPV genotypes 16, 18, and/or 45. This information together with physician's assessment of cytology history, other risk factors and professional guidelines, may be used to guide patient management.</p>
IGF-2, LC/MS	<p>IGF-2 measurements are useful in the diagnosis of growth disorders, as an adjunct to IGF-1.</p>

Recent Publications

Quest scientists are always looking to enhance their knowledge across a broad range of specialties. Here we show some of their recent publications.

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