Welcome to the first quarterly Quest Diagnostics Clinical Trials Scientific Affairs newsletter. The Scientific Affairs Team is composed of scientific professionals who leverage the scientific expertise in Quest Diagnostics to provide consultative and advisory services for our sponsors, from pipeline biomarker analysis and companion diagnostics, to late phase clinical trials. If you have any questions, please contact us at: clinical.trials@questdiagnostics.com.

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Urinary Biomarkers of Drug-Induced Nephrotoxicity
Kamala K. Maddali, D.V.M., Ph.D.
Harvey W. Kaufman, M.D., MBA, FCAP

Key Points
Nephrotoxicity is a major reason for safety-related failures in drug development, often causing drugs to be withdrawn during late stages (phase 2/3) of clinical development or even post-marketing.

The U.S. FDA and other regulatory agencies have qualified seven analytes as acceptable biomarkers of drug-induced nephrotoxicity in rat preclinical studies.

The availability of accurate early biomarkers could greatly reduce the likelihood of novel drugs failing due to nephrotoxicity at late stages of development or post-marketing.
Drug-induced Nephrotoxicity

The kidneys receive approximately 22% of the cardiac output. Key functions of the kidneys include waste removal, maintaining salt and acid balance in the blood, and hormone production. Each kidney contains about 1 to 2 million nephrons, which concentrate substances from the blood for excretion in urine. Filtration in the nephron begins in the glomeruli; nutrients, water, and salts are then reabsorbed as the filtrate flows through the proximal tubule, loop of Henle, distal tubule, and finally into the collecting duct.

The kidneys play a central role in excretion and detoxification of many drugs, some of which reach very high concentrations in parts of the nephrons. Thus, drug-induced nephrotoxicity and kidney injury are anticipated. Drug-induced kidney injury can be acute or chronic and can arise from pre-renal, intrarenal, or post-renal (vascular, tubular, glomerular, or interstitial) assaults. Nephrotoxicity is a relatively common cause of acute kidney injury (AKI), a critical complication defined as a rapid decline in glomerular filtration rate (GFR; a measure of renal function).1

Mechanisms of kidney injury vary by drug and may include inflammation, changes in glomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy; Table 1 lists a few examples of drug classes that can cause kidney damage, along with sites and mechanisms of injury.2 Understanding how and where medications exert their nephrotoxic effects can provide useful insight for the development of drugs with therapeutic benefits and reduced adverse effects. This information can also help in the development of biomarkers for sensitive and specific detection of drug-induced nephrotoxicity.

Table 1. Classes of Medications that Can Cause Kidney Damage

<table>
<thead>
<tr>
<th>Drug class</th>
<th>Mechanisms or Site of Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diuretics</td>
<td>Inflammation; acute interstitial nephritis (thiazides) and crystal nephropathy (e.g., triamterene)</td>
</tr>
<tr>
<td>Immunosuppressants</td>
<td>Interstitial fibrosis (e.g., tacrolimus)</td>
</tr>
<tr>
<td>Antiplatelet agents</td>
<td>Thrombotic microangiopathy (e.g., clopidogrel)</td>
</tr>
<tr>
<td>Antivirals</td>
<td>Crystal nephropathy (e.g., foscarnet); tubule cell toxicity (e.g., tenofovir)</td>
</tr>
<tr>
<td>Antineoplastic agents</td>
<td>Distal tubules (e.g., cisplatin) and proximal tubules (e.g., ifosfamide)</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Proximal tubule endothelial cells (e.g., aminoglycosides); multiple segments of the renal tubules (e.g., amphotericin B)</td>
</tr>
<tr>
<td>Radiocontrast agents</td>
<td>Renal tubule epithelial cells</td>
</tr>
</tbody>
</table>

Sources:
aac.asm.org/content/43/5/1003.full
emedicine.medscape.com/article/1925868-overview
aafp.org/afp/2008/0915/p743.html

Need for Early Markers of Nephrotoxicity in Clinical Drug Development

In the clinical setting, drug-induced nephrotoxicity may account for approximately 20% of acute renal failure episodes.2 In the pharmaceutical setting, nephrotoxicity is a major reason for safety-related failures in drug development, often causing drugs to be withdrawn during late stages (phase 2 or 3) of clinical development or even post-marketing.3-6 Given the expense of drug development and the morbidity associated with kidney injury, financial and human costs can rise dramatically if nephrotoxicity is not discovered until the clinical development stage or later.6

Late detection of drug-induced nephrotoxicity has been due in part to reliance on traditional markers - serum creatinine and urea nitrogen - to monitor kidney function during preclinical and clinical development. Unfortunately, these
markers are relatively insensitive, are dependent upon age, gender, and ethnicity, and do not provide evidence of the mechanism or site of kidney injury. Moreover, they do not provide timely information, as they often show little appreciable change until the kidneys have sustained extensive damage and function has declined by more than 50%. Reliance on these markers can thus have an adverse impact on timely clinical trial designs and decisions for novel therapies.

Clearly, there is a need for a set of early, sensitive, specific, and accessible biomarkers that are produced at specific sites of nephron injury. Ideally, these markers would provide crucial information about the site as well as the mechanism(s) of drug-induced nephrotoxicity. Incorporating such biomarkers should provide more discriminatory tests of toxicity to eliminate compounds earlier in the development process.

**Regulatory Efforts**

The development of novel kidney safety biomarkers has been led by the Predictive Safety Testing Consortium (PSTC) Nephrotoxicity Working Group of the Critical Path Institute (C-Path). The nephrotoxicity working group members include over 200 scientists from leading pharmaceutical companies and non-profit research organizations, along with advisors from the Food and Drug Administration (FDA) and the European Medicines Agency (EMEA).6,7 Based on the submission of drug toxicity and biomarker performance analyses by the PSTC, the FDA and EMEA qualified seven kidney safety biomarkers for voluntary use in nonclinical studies in rats (Table 2).6 Table 3 summarizes the site specificity of these markers. With the exception of TFF-3, the qualified kidney biomarkers have been reported to outperform the current standard kidney biomarkers (serum creatinine and urea nitrogen).6,7 Efforts to qualify these biomarkers for human clinical studies are underway.

### Table 2. Seven Biomarkers Qualified for Use in Renal Safety Studies in Rats

<table>
<thead>
<tr>
<th>Marker</th>
<th>Comments</th>
</tr>
</thead>
</table>
| Albumin     | • Biomarker of kidney complications involving diabetes. Increased levels suggest glomerular dysfunction.  
• Biomarker of functional disturbance of tubular epithelium. Small quantities are filtered and efficiently reabsorbed by tubular epithelium. |
| ß-2 microglobulin | • Biomarker of renal tubular function and neoplastic, inflammatory, and infectious conditions. Primarily filtered by the glomeruli and reabsorbed by the proximal tubular cells. |
| Clusterin   | • Sensitive kidney/urinary marker of AKI in preclinical models. Plays an important role in tubular epithelium regenerative repair response. |
| Cystatin C  | • Functional biomarker of glomerular filtration and tubular reabsorption. Elevated urinary levels reflect tubular dysfunction.  
• Glomerular damage yields protein overload to lumen to prevent efficient tubular reabsorption process of cystatin C from lumen. |
| KIM-1       | • Associated with regeneration/dedifferentiation of proximal tubule epithelial cells after ischemic/toxic injury. Sensitive and specific biomarker of AKI. |
| TFF-3       | • Biomarker of dysfunction of glomeruli or proximal tubular reabsorption. TFF-3 is cytoprotective; decreased levels reduce cellular maturation signaling, allowing dedifferentiation.  
• Found in animals only; there is no human analog. |
| Total urinary protein | • General indicator of glomerular damage and nephrotic syndrome. |
Future Directions

The availability of qualified biomarkers of nephrotoxicity in rat kidney safety studies is a large step on the path to timely detection of drug-induced nephrotoxicity during nonclinical and early clinical drug development. Use of these qualified biomarkers as part of an early kidney safety biomarker strategy could enable more accurate and prompt identification of drug-induced nephrotoxicity.

Following the discovery, validation, and finally regulatory acceptance of these biomarkers, they have proven to be of great value in early detection of nephrotoxicity. Incorporation of these kidney safety biomarkers in early and late phase clinical trials, based on the results from preclinical studies, will be relevant development of investigational drugs. Further, voluntary submission of the clinical trial data to the FDA, mimicking the preclinical kidney toxicity PSTC initiative, will be valuable for the wider acceptance of these kidney safety biomarkers for future clinical trial programs.

Limitations

Caution should be exercised in the use and interpretation of tests for qualified biomarkers. According to the FDA, "Biomarkers being considered for qualification are conceptually independent of the specific test performing the measurement. ... FDA clearance of a testing device for marketing does not imply that the biomarker it measures has been demonstrated to have a qualified use in drug development and evaluation. Additionally, qualification of a biomarker does not automatically imply that a specific test device used in the qualification process for a biomarker has been reviewed by FDA and cleared or approved for use in patient care."8

Table 3. Site Specificity of the Qualified Biomarkers of Drug-Induced Kidney Toxicity

<table>
<thead>
<tr>
<th>Marker</th>
<th>Site Specificity</th>
<th>Site Specificity</th>
<th>Site Specificity</th>
<th>Site Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>Proximal tubules</td>
<td>Distal tubules</td>
<td>Glomeruli</td>
<td>Collecting duct</td>
</tr>
<tr>
<td>B2M</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Clusterin</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>KIM-1</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>TFF-3a</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Total protein</td>
<td></td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
</tbody>
</table>

8-2M indicates β-2 microglobulin; KIM-1, kidney injury molecule 1; TFF-3, trefoil factor 3.

Animal biomarker only—no human analog

Conclusions

Implementation of a kidney safety biomarker strategy for early detection of nephrotoxicity during drug development offers great opportunity to identify drugs that cause
nephrotoxicity earlier in the development process.\textsuperscript{9,10,11} Although existing markers are now qualified only for preclinical studies in rats, many clinical trial studies are underway to qualify markers for wider use of these biomarkers for predicting outcomes related to drug induced nephrotoxicity.

Role of Quest Diagnostics Central Laboratory

Quest Diagnostics has a proven track record as a leader in improving the diagnosis of kidney disease:

- Leadership in introducing the MDRD (Modification of Diet in Renal Disease) Study equation to calculate the estimated GFR (eGFR).
- Among the first laboratories to introduce the improved CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation to report eGFR.
- Revised the upper limit of the serum creatinine reference ranges to match an eGFR of 60 mL/min/1.73m\(^2\) in older adults.
- Ongoing work with the National Kidney Foundation (NKF) has placed the company at the forefront of physician education.
- For a decade, Quest Diagnostics has provided laboratory testing services for the NKF Kidney Early Evaluation Program (KEEP\textsuperscript{\textregistered}).

As an active participant in the creation of central laboratory solutions, our responsibility is to design and implement quality go/no-go decision-making biomarker assays for clinical drug development programs. Our extensive test selection offers the flexibility to create custom multiplex and singleplex biomarker panels representing both conventional and novel biomarkers. The contribution of central laboratories in implementing validated novel biomarkers in a harmonized and standardized manner is vital to minimize any potential errors that could affect data interpretation. The ultimate goal is to maintain low costs for global clinical trial execution and support the introduction of better and safer drugs into clinical use.

References

Authors:

Harvey W. Kaufman, M.D. is Senior Medical Director, Office of Medical Affairs at Quest Diagnostics where he has been employed since 1992 serving a variety of roles including first Chief Laboratory Officer, first Six Sigma Leader, and medical director, International. He is a member of the board of the Quest Diagnostics Foundation. Dr. Kaufman earned M.D. from Boston University School of Medicine, and M.B.A. from New York University Leonard N. Stern School of Business. He is board certified in Anatomic and Clinical Pathology and Chemical Pathology. Dr. Kaufman is an original and current member of the National Kidney Disease Education Program Laboratory Steering Group, served on the New Jersey and serves on the New York chronic kidney disease task forces, and in 2004 received the Niagara Health Quality Coalition Best Of Best Health Care Innovation Award for “revolutionizing kidney disease diagnosis nationally.”

Kamala Maddali, DVM, PhD is a Director of Scientific Affairs, Clinical Trials at Quest Diagnostics. Dr. Maddali formerly worked as a Scientist at both Huntingdon Life Sciences and Merck Research Laboratories; bringing 12 years of pharma/CRO industry experience. Most recently, she held the position of Associate Director, Biomarkers and Companion Diagnostics at Quintiles. In this role, Dr. Maddali acted as a scientific advisor for clinical biomarkers and companion diagnostic strategies for clinical trials. Her role also supported the sales teams globally with scientific and technical support and advice to clients on biomarker capabilities and scientific solutions. In her current role, Dr. Maddali provides timely consultation to internal and external customers regarding scientific and technical information as well as laboratory or other data/information analysis. Also provides support to aid in enhancing Quest Diagnostics goals in US and globally, by providing technical training and customer education/technical support on test choices/methods and study protocol design, as well as scientific consultation with pharmaceutical doctoral researchers and scientists. Dr. Maddali is a current and active member of AAPS Biomarkers in Translational Medicine focus group.

The BRCAvantage™ Test for Breast Cancer Clinical Trial Testing

Breast cancer is the most common cancer diagnosed among women in the United States. It accounts for nearly 1 in 3 cancers among women. It is also the second leading cause of cancer death among women after lung cancer. Hereditary Breast cancer accounts for about 5% to 10% of female breast cancer and 4% to 40% of male breast cancer. The most common cause is mutations in the BRCA1 and BRCA2 genes. Mutations in these genes also account for up to 15% of all ovarian cancers. BRCA1 and BRCA2 (breast cancer susceptibility genes 1 and
2) are tumor suppressor genes located on chromosomes 17 and 13, respectively. These genes encode proteins that are involved in DNA repair. Mutations inhibit the DNA repair function, increasing the risk for certain cancers. \textit{BRCA1} and \textit{BRCA2} mutations are primarily linked to hereditary cancer of the breast, ovary, and fallopian tube and primary papillary serous cancer of the peritoneum. They are also linked to pancreatic cancer, male breast cancer, prostate cancer, and melanoma.

**Early Predictive/Cost Effective Tool for Clinical Trial Recruitment**

Recent studies have suggested a link between \textit{BRCA} mutations and triple-negative disease. Although chemotherapy is effective in triple-negative disease, more targeted therapies are being developed. Antiangiogenic agents such as bevacizumab have demonstrated efficacy across subtypes. More recently, poly (ADP-ribose) polymerase inhibitors appear to take advantage of the concept of synthetic lethality, or dual pathway inhibition, in attacking triple-negative and \textit{BRCA}-associated tumors. These and other studies in triple-negative disease will help us to better identify effective treatment options and improve outcomes in these patients.

The BRCAvantage™, Comprehensive test can be used as a “patient stratification tool” to determine if a patient is at increased risk of \textit{BRCA1}- and \textit{BRCA2}-associated cancers. Such testing is recommended in multiple guidelines. Individuals who test positive for a \textit{BRCA1} or \textit{BRCA2} mutation have several options to decrease their risk of cancer. These include increased surveillance, prophylactic mastectomy and/or salpingo-oophorectomy, chemoprevention and targeted therapy clinical trials.

**Individuals Suitable for Testing**

Men and women at high risk for \textit{BRCA1}- and \textit{BRCA2}-associated cancer.

**Method**

- Quest Diagnostics provides highly sensitive and specific \textit{BRCA} testing. Quest Diagnostics uses two separate next generation sequencing platforms in order to minimize false positives and false negatives. Other current market \textit{BRCA} mutation testing includes testing performed by sequencing of \textit{BRCA1} and \textit{BRCA2} by Sanger sequencing. Other entrants perform next-generation sequencing, but they only use one platform.
- The BRCAvantage service, including MLPA technology used for rearrangement testing, was developed and validated in a manner wholly compliant with federal law and regulations, the Clinical Laboratory Improvement Amendments, and our own high standards of quality.
- Clinical validation studies demonstrated a rate of 100% sensitivity and specificity.

**The Quest Diagnostics Advantage**

Quest Diagnostics Clinical Trials has supported over 590 oncology related clinical studies in the last 10 years. Our biomarker services include development and utilization of novel/proprietary assays. Testing services include genomics, molecular diagnostics and proteomics. Our testing services can facilitate patient screening and enrollment as well as assessing drug response.

For more information regarding our assays, capabilities, or how we can support your clinical trial, please visit our website at: Questdiagnostics.com/home/companies/clinical-trials.
**Latest News**

**Quality Improvement Program**
At Quest Diagnostics we take quality seriously and are continuously striving to improve our processes in line with our ISO 9001 certification. This internationally recognized standard is aimed at achieving customer satisfaction by setting out what needs to be in place in order to consistently meet customer requirements and ensuring that a quality-led philosophy is maintained.

Our Quality Improvement (QI) teams in the UK and the US aim to improve our internal working in order to achieve global consistency and constantly strive to improve the quality of service to our customers. With a team of four, they carry out a rolling program to monitor our processes in the laboratory as well as bringing proactive solutions for any issues identified. The QI teams work with our scientists to implement these improvements across our global network of clinical trials laboratories. They also provide interactive educational tutorials on a range of topics such as documentation technique, pipette calibration and humidity monitoring requirements, both in local classroom sessions and via web meetings to our Alliance laboratories such as China and Singapore.

**New Platform to Meet Client Needs**
The Quest Diagnostics Clinical Trial Laboratory in the UK recently purchased an Advia 2120i, a fully automated CBC analyzer, in order to support the specific test requirements of one of our clients. The Advia, which will be used alongside our LH750s, is able to produce all the ‘standard’ CBC parameters, and in addition has the capability to generate specific ‘research’ morphology flags such as % hypochromia; % hyperchromia; %macro, %micro, micro/hypo ratio, PDW (platelet distribution width), and RBC fragments.

**Increased Support in China**
In order to better support our Clients’ needs in China, Quest Clinical Trials has recently hired a new Sales Account Executive – Ms Yingli Guo. She will be responsible for business development and revenue target in China. Before joining Quest Diagnostics Clinical Trials, Yingli was the marketing manager of Thermo Fisher Scientific since 2009, where she focused on the Biologics segment, with main responsibilities including market analysis, mapping out market size, market share, competitive profile and market growth, focus accounts maintenance and new potential focus account development.

Prior to 2009, Yingli worked in Shanghai Genomics Inc as department head and project leader for many outsourcing projects with major global pharmaceutical companies.

Yingli has a Masters Degree in Biochemistry and Molecular Biology.

**Recent Publications**
Quest Scientists are always looking to enhance our knowledge across a broad range of specialisms. Here we show some of our recent Publications:


Newest Tests

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

1. **HIV-1 Genotype (RTI, PI, Integrase Inhibitors)**
   Identify drug resistance mutations in HIV-1 patients failing antiretroviral regimens containing RT, PR or Integrase inhibitors.

   **Method:** HIV-1 RNA extraction followed by reverse transcription and PCR amplification. DNA sequence is determined and mutations predictive of drug resistance are reported, using the AB3730 DNA capillary sequencer.

2. **Hepatitis C Viral RNA NS3 Genotype**
   This assay may be used to detect boceprevir and telaprevir resistance-associated NS3 mutations in NS3 protease inhibitor treatment-experienced patients.

   **Method:** This assay amplifies and sequences the HCV Genotype 1 NS3 gene protease domain codons 1-181 and reports mutations at positions associated with protease inhibitor resistance, using the AB3730 DNA capillary sequencer.

3. **Influenza A and B Virus with Subtyping**
   This test is used to determine the presence of influenza A or B viral RNA in a patient’s specimen, and to differentiate among possible influenza A virus subtypes.

   **Method:** The assay is composed of two principal steps: (1) extraction of nucleic acids from patient specimens and (2) one-step reverse transcription (RT)-PCR amplification and detection with probes detecting paninfluenza, A virus, influenza B virus, influenza A H1N1 (2009) virus, seasonal influenza A H3N2, virus and influenza A H3N2v virus.
4. Secretory Phospholipase A2 (sPLA2)
Superfamily of enzymes that hydrolyze the ester bond of phosphoglycerides at the sn-2 position to release free fatty acid and lysophospholipids.

Production of sPLA2-IIA is up-regulated in response to pro-inflammatory compounds such as interleukin (IL)-1β, IL-6, tumor necrosis factor-α, interferon-γ, and oxidized low density lipoprotein (LDL). The potential value of sPLA2-IIA mass might be in predicting the risk of cardiovascular diseases (CVD) alone or in conjunction with other biomarkers. The involvement of sPLA2-IIA in various atherogenic mechanisms would make sPLA2-IIA a promising treatment target in patients with atherosclerosis.

Method: Microwell-plate double antibody sandwich immunoassay.

5. 14-3-3 ETA protein
14-3-3 ETA protein family consists of seven highly conserved dimeric proteins: alpha/beta, delta/zeta, epsilon, gamma, eta, tau/theta, and sigma.

14-3-3 ? (eta) is normally an intracellular synovial protein, and only in the disease state it is released into the extracellular space. The extracellular 14-3-3 eta protein has been described as a novel biomarker associated with joint damage in rheumatoid and psoriatic arthritis. The 14-3-3 eta measurement complements RF and anti-CCP tests and improves diagnostic, prognostic and therapy monitoring applications. Additionally, 14-3-3 eta has been shown to be a marker of early RA that may contribute to pathological processes of the disease.

Method: Microwell-plate double antibody sandwich immunoassay

6. BRAF V600 mutation
The majority of BRAF mutations in melanoma occur in codon 600 with the predominant mutation at codon 600 being the V600E mutation (GTG → GAG). Recent data from clinical trials show promising results with the BRAF inhibitor, demonstrating complete or partial tumor regression in 81% of patients with metastatic melanoma harboring the V600E BRAF mutation. The BRAF V600E mutation test is intended to be used as an aid in selecting melanoma patients with the BRAF V600E mutation for potential treatment.

Sensitivity: Our studies demonstrate that this assay has an approximate sensitivity of detecting 5% mutation-bearing cells in a mixed population.

Method: The cobas® 4800 BRAF V600 Mutation Test is based on two processes: (1) manual specimen preparation to obtain genomic DNA from formalin-fixed, paraffin-embedded tissue (FFPE); (2) PCR amplification and detection target DNA using a complementary primer pair and two oligonucleotide probes labelled with different fluorescent dyes.

7. Lung cancer ALK rearrangement
Non-small cell lung cancer is the leading cause of cancer death worldwide. With a 5-year morbidity rate of 85 - 95%, there is a pressing need for improvement in identifying patients most likely to respond to specific treatments. Detects chromosome 2p23 rearrangements. Rearrangement of the ALK locus on 2p23 has been implicated in the development of NSCLC.

The ALK gene codes for a transmembrane glycoprotein with tyrosine kinase activity. Tyrosine kinase inhibitors have been demonstrated to reduce lung cancer cell proliferation, resulting in suppression of tumor growth. The therapeutic efficacy of inhibiting ALK in tumors that were selected by ALK positivity using FISH has been demonstrated in an early-phase clinical trial of a small molecule inhibitor of the ALK tyrosine kinase.

Method: Fluorescence in situ hybridization (FISH): allows the visualization of specific chromosome nucleic acid sequences within a cellular preparation. Specifically, FISH involves the precise annealing of a single-stranded, fluorophore-labeled DNA probe to complementary target sequences. The hybridization of the probe with the cellular DNA region is visible by direct detection using fluorescence microscopy.

8. ROMA (Risk of Ovarian Malignancy Algorithm, based on HE4 EIA and menopausal status)
Pre-surgical evaluation of ovarian tumours should include
menopausal status, physical examination, transvaginal ultrasonography, CA 125 concentration, and family history of breast or ovarian cancer in a first-degree relative.

ROMA can be used as a supplement to the standard presurgical evaluation to assess the likelihood that an ovarian mass is malignant in women whose pre-surgical assessment did not indicate malignancy, and to assess the need to refer the patient to a gynecologic oncologist for treatment.

Method: Combines the results of human epididymis protein 4 (HE4) enzyme immunometric assay (EIA), ARCHITECT CA 125 II™, and menopausal status to generate a single numerical score that correlates with the likelihood of malignancy being seen at surgery.

9. TERC: Cervical Cancer
The new Quest Diagnostics test is designed as an adjunct to conventional Pap and HPV tests, and is performed on residual samples from Pap tests.

It detects abnormal changes to the TERC gene and chromosome 3 to provide a risk assessment of progression to cervical cancer in women who receive indeterminate Pap and/or HPV test results.

TERC results help categorize risk in abnormal Pap tests prior to colposcopy, a procedure to visually inspect cellular changes.

Women with the highest risk result may benefit from additional cervical biopsies at colposcopy and more aggressive monitoring and treatment, while women with low-risk TERC and HPV results may be less likely to undergo unnecessary follow-up colposcopy and other procedures.

Method: Fluorescence in situ hybridization (FISH): allows the visualization of specific chromosome nucleic acid sequences within a cellular preparation. Specifically, FISH involves the precise annealing of a single-stranded, fluorophore-labeled DNA probe to complementary target sequences. The hybridization of the probe with the cellular DNA region is visible by direct detection using fluorescence microscopy.

10. Myelodysplastic Syndrome (MDS)
Mutations, Sequencing
next-generation sequencing panel used in the diagnostic workup of patients with persistent cytopenia(s) and myelodysplastic syndrome (MDS). The assay can be used to identify mutations as clonal markers, which our studies demonstrate are present in the majority of MDS cases with increased blasts and approximately 30% of cytopenias referred for workup in adults older than 50 years without increased blasts.

Method: This assay is a next-generation sequencing (NGS), DNA-based assay that targets the frequently mutated areas of 7 genes (ASXL1, EXH2, IDHI, IDH2, KRAS, NRAS, and TET2)

11. Melanoma, Chromosomal Microarray, ClariSure® Oligo-SNP
This assay detects alterations in copy number or allelic imbalance.
It can be used in the differential diagnosis of melanocytic lesions (e.g., classifying indeterminate lesions as benign or malignant).

Method: The assay is performed using DNA extracted from FFPE samples. Oligonucleotide probes are attached to beads and assembled into the microwells of the BeadChip.

The DNA samples are denatured followed by uniform whole genome amplification. The amplified DNA is fragmented, precipitated, resuspended in hybridization buffer, and hybridized overnight to the BeadChip. Following washing, single-base extension and staining of the oligos on the chip, using the captured DNA as a template incorporates detectable labels and determines the genotype call for the sample. The Illumina iScan System scans the BeadChip, using laser to excite the fluorophore of the single-base extension product on the beads. The scanner records high-resolution images of the light emitted from the fluorophores.

12. Chromosomal Microarray, Oncology, ClariSure® Oligo-SNP
This assay detects alterations in copy number or allelic imbalance.
It can be used in the differential diagnosis of solid tumors (e.g., classifying indeterminate lesions as benign or malignant).
Featured Case Study: HIV1 Genotypic Tropism Assay

Background and Challenges:
A client wishing to perform HIV Tropism testing was using the enhanced sensitivity Tropism Assay (ESTA).

Although considered to be the current ‘Gold Standard’, this phenotype test is expensive, labour intensive and usually requires several weeks for reporting.

Alternative assays are needed – but ideally have to have been clinically validated to support use in a Clinical Trial.

Solution
Quest had carried out a clinical validation of a genotypic tropism test that used triplicate population sequencing (TPS) with reflex to ultradepth sequencing (UDS), comparing the results obtained to the ESTA.

Consequently, the Quest genotype assay was included in the trial as a comparison to the standard phenotype assay.

Benefits to Client
Faster turn-around time (TAT): result obtained much quicker than using ESTA, allowing quicker enrollment
Cost effective: budget savings that could be utilized for other aspects of a study
Quality results: Quest’s genotype assay was able to identify more subjects with the non-R5 virus, avoiding enrollment of potential non-responders.

Method: The assay is performed using DNA extracted from FFPE samples. Oligonucleotide probes are attached to beads and assembled into the microwells of the BeadChip.

The DNA samples are denatured followed by uniform whole genome amplification. The amplified DNA is fragmented, precipitated, resuspended in hybridization buffer, and hybridized overnight to the BeadChip. Following washing, single-base extension and staining of the oligos on the chip, using the captured DNA as a template incorporates detectable labels and determines the genotype call for the sample. The Illumina iScan System scans the BeadChip, using laser to excite the fluorophore of the single-base extension product on the beads. The scanner records high-resolution images of the light emitted from the fluorophores.

13. Multiplex Cytokine markers: IL-6, TNF-alpha, IL-12p-70, IL-8, IL-10, IL-1beta (6-plex)
The interest in Proinflammatory cytokines as markers of various pathological processes continues. Having assays that can perform an analysis of a single sample for many analytes results in a saving of cost, testing time, and sample volume.

Method: electrochemiluminescent immunoassay on the MSD platform. Capture antibodies are coated in arrays in each well of a 96-well carbon electrode plate surface. Specific analytes bind to the capture antibodies, and tagged second antibody completes the sandwich process. Addition of the MSD read buffer elicits a chemical reaction detected by the plate reader.