Clinical Trials

Next Generation Sequencing (NGS) Technology in Oncology Drug Development
Onikepe Adegbola, MD PhD

Overview of NGS technology
In the decade since the completion of the human genome project, DNA sequencing technology has changed considerably. The commercial availability of the first massively parallel pyrosequencing platform in 2005 led to a new era of high-throughput genomic analysis, now referred to as next-generation sequencing (NGS). NGS technologies use a fundamentally different approach to sequencing, in which samples are sequenced massively in parallel with ever-decreasing costs promised to reach the $1000 genome mark this year.

In Sequence’s 2013 year-end annual assessment observed that four major companies offered NGS technologies available for purchase: Illumina, Life...
Technologies, Roche, and Pacific Biosciences. Additionally, there is great market anticipation of Oxford Nanopore Technologies’ first commercially available machine. Original NGS technologies (second-generation), including Illumina’s HiSeq, Life Technology’s SOLiD and Ion Torrent, and Roche’s 454 methods, all utilize attachment of a genomic library to a solid surface, followed by amplification, and a sequencing technology involving alternating phases of nucleotide incorporation with signal detection. These technologies’ greatest limitations are short read length and amplification bias, which they attempt to overcome by massively parallel sequencing. Illumina, which sequestered >70% of the sequencing market in 2013, announced its newest machine, the HiSeq XTen, will sequence 600 gigabases of sequence a day with 150 bp reads.

Third-generation NGS technologies, including Pacific Biosciences SMRT technology and Oxford Nanopores strand sequencing, are single-molecule methods with real-time nucleotide detection. The PacBio RS real-time detection of labeled nucleotide incorporation allows for long read-length capabilities (~10 kb raw reads) not possible in second-generation NGS technologies, but reported high error rates of ~15% have limited the technology’s usefulness. Nanopore technology is a promising technology which involves passing a single strand of DNA through a monitored, protein pore that emits a specific electronic frequency as each type of base passes through. If this technology meets the anticipated hype, it will result in a real-time, long, single molecule sequencing technology with no nucleotide incorporation bias. The following discussion examines how NGS technologies currently benefit oncology clinical trials, and what challenges remain to their full implementation.

**Benefits of NGS in Oncology Drug Development**

**Genomics in the design of clinical trials:** Many new anticancer drugs target proteins encoded by genes with driver somatic mutations, thus exhibiting efficacy only in a subgroup of patients whose tumors harbor these mutations. This has resulted in changes to standard clinical trial design to include a study population with patients whose tumors carry a particular mutation, set of mutations, or other biomarker, increasing the potential of response to targeted therapy. With the costs of NGS dropping precipitously, the complete sequencing of cancer genomes and transcriptomes would be routinely used in oncology clinical trials in the future to reveal genomic and epigenomic changes. NGS streamlines testing to identify all novel genomic changes, including gene mutations, copy number changes, and translocations, instead of running separate assays that eat up costs, time, and precious sample DNA. NGS allows differentiation of a variety of cancer scenarios through its massively parallel testing capabilities. A single sequencing run can evaluate a tumor’s primary, metastatic, or relapsed state by identification of biomarkers in both the current and previous tumor (even if embedded in FFPE).

NGS enables high-throughput analyses of the transcriptome and epigenome. Transcriptome analysis using NGS RNA-seq has identified cancer-causing protein fusion events undetectable at the genomic level. NGS transcriptome analysis can help in predicting drug response, an emerging strategy that identifies active pathways in a given tumor cell line. NGS based ChiP-seq
analysis identified patients for epigenetic drug clinical trials, such as combination trials on DNA methylation inhibitors and histone deacetylase inhibitors.

**Personalized cancer treatment:** Gene expression signatures obtained using NGS technologies could be used for prognosis and hence to influence therapeutic intervention. NGS has been successfully employed to identify novel mutations in a variety of cancers, including bladder cancer, colon cancer, renal cell carcinoma, small cell lung cancer, prostate cancer, acute myelogenous leukemia, and chronic lymphocytic leukemia. In recent times, NGS technology has been used for personalized treatment of cancer, e.g. in the treatment of pancreatic cancer, detection of EGFR deletions in nonsmall cell lung cancer, and detection of the PML-RAR fusion gene in acute promyelocytic leukemia. In future, it will be possible to use NGS technologies to obtain a comprehensive molecular profile for a patient within a clinically acceptable timeframe and cost.

**Role of NGS in clinical trials:** NGS has numerous roles in oncology clinical trials. For instance, in a single agent trial, NGS can be used to identify all relevant genetic aberrations and this data can be utilized to identify genomic biomarkers for response and/or primary resistance. In a longitudinal or targeted therapy study, along with identifying biomarkers responsible for response and primary and acquired resistance, NGS can aid in identifying biomarkers for rational drug combination. In a multiple simultaneous clinical trial setting, NGS can increase the likelihood of identifying eligible patients since all key genomic aberrations are tested upfront providing the investigator with all of the relevant information about clinical trial participants which will enable improved research opportunities. In an unsuccessful clinical trial, NGS data can be assessed to explain unexpected clinical trial outcomes, providing insight into the mechanism of drug effect and possible reasons for intergroup differences in response rate.

**Development of new therapeutics:** Various inhibitors targeting proteins encoded by mutated cancer genes (e.g. imatinib [Gleevec], a potent inhibitor of the Abelson kinase for chronic myeloid leukemia) have been successfully developed. Large-scale cancer genome studies, such as the International Cancer Genome Consortium and the Cancer Genome Atlas, are applying NGS to tumors from 50 different cancer types to generate more than 25,000 cancer genomes at genomic, epigenomic, and transcriptomic levels to generate a complete catalogue of oncogenic mutations, some of which may prove to be new therapeutic targets.

**Acquired resistance to therapy:** Genomic changes, ranging from point mutation within the gene encoding the protein to which a drug is targeted (e.g. EGFR mutations in non-small-cell lung cancer) to amplification of an entirely different cancer gene (e.g MET in non-small-cell lung cancer) can result in tumors that are resistant to drugs. Deep sequencing of cancer genomes with NGS enables detection of small numbers of resistant cells, providing valuable information on combination-treatment strategies that might minimize the chances of the resistant clones.

**Susceptibility to cancer:** Genome-wide association studies, which compare the prevalence of hundreds of thousands of inherited variants between large series of patients with disease and control subjects, have resulted in the identification of many new DNA variants conferring
susceptibility to several different types of cancer, including cancer of the breast, prostate, colon and rectum, lung, and testis, along with chronic lymphocytic leukemia and other cancers.

**Detection of circulating cancer DNA by NGS:** Rare mutations in circulating DNA have long been used to detect somatic mutation for cancer diagnosis and management.

NGS offers a potentially cost-effective method to detect and measure the allele frequency and other tumor gene mutations in plasma, due to the ability to sequence large regions of circulating DNA.

**NGS platforms under development**

Several unique single-molecule DNA sequencing technologies are currently under development (Tables 1.A-1.D).

### Table 1.A Fluorescence-based single-molecule sequencing

<table>
<thead>
<tr>
<th>Developer</th>
<th>Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pacific BioSciences</td>
<td>Single-molecule real time (SMRT) DNA sequencing technology which performs single-molecule sequencing by identifying nucleotides which are phospholinked with distinctive colors</td>
</tr>
<tr>
<td>Visigen Biotechnology</td>
<td>Total internal reflectance fluorescence (TIRF) technology to measure the time-dependent fluorescent signals emitted from each single DNA strand in parallel based on fluorescence resonance energy transfer (FRET)</td>
</tr>
<tr>
<td>U.S. Genomics</td>
<td>Fluorescence-based single-molecule sequencing platform, in which short-universal probes are hybridized to their complementary DNA fragments and proprietary microfluidics stretch the DNA strand into full contour length</td>
</tr>
<tr>
<td>Genovoxx</td>
<td>AnyGene technology for fluorescence-based single-molecule sequencing by monitoring the sequential addition of each single nucleic acid during DNA synthesis</td>
</tr>
</tbody>
</table>

### Table 1.B Nano-technologies for single-molecule sequencing

<table>
<thead>
<tr>
<th>Developer</th>
<th>Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxford Nanopore Technologies</td>
<td>Exonuclease sequencing technology that combines a protein nanopore bioengineered with a covalent attachment of a cyclodextrin molecule to the inside of its surface with an exonuclease for the sequential identification of DNA bases as the processing enzyme passes through the nanopore</td>
</tr>
<tr>
<td>Nabsys</td>
<td>Nanopore 6-mer oligonucleotides hybridization mapping technology in which electrically addressable nanopore arrays in a solid state can sequence DNA strands in parallel</td>
</tr>
<tr>
<td>BioNanomatrix</td>
<td>Nano Analyzer platform for single-molecule sequencing that uses a small nanochannel fluidic chip for long DNA molecule sequencing</td>
</tr>
<tr>
<td>GE research</td>
<td>“Closed Complex” where stable complexes are formed between primed single DNA molecules, polymerase, and nucleotides on a solid surface in a microfluidic system</td>
</tr>
<tr>
<td>Roche and IBM</td>
<td>“DNA transistor” sensor which is potentially capable of recording nucleotide sequence as a single strand of DNA</td>
</tr>
<tr>
<td>LingVitae (Digital Sequencing)</td>
<td>New single-molecule sequencing platform using “design polymers” technology to slow down the translocation of DNA strands through the nanopore for better read quality during nano-sequencing</td>
</tr>
<tr>
<td>Base4 Innovation</td>
<td>Technology to combine Surface Enhanced Raman spectroscopy (SERS) with nano-resolution to interpret individual bases in a single strand of DNA with their patented nanostructure arrays</td>
</tr>
</tbody>
</table>
### Table 1.C Electronic detection for single-molecule sequencing

<table>
<thead>
<tr>
<th>Developer</th>
<th>Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reveo</td>
<td>Technology to stretch out DNA molecules on conductive surfaces for electronic base detection</td>
</tr>
<tr>
<td>Intelligent Biosystems</td>
<td>Electronic detection approach to develop a platform which will allow for high speed and high sensitivity single-molecule analysis with decreased background noise</td>
</tr>
</tbody>
</table>

### Table 1.D Electron microscopy for single-molecule sequencing

<table>
<thead>
<tr>
<th>Developer</th>
<th>Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>LightSpeed Genomics</td>
<td>Microparticle approach by capturing sequence data with optical detection technology and new sequencing chemistry from a large field of view to reduce the time-consuming sample and detector rearrangement</td>
</tr>
<tr>
<td>Halcyon Molecular</td>
<td>DNA sequencing technology by atom-by-atom identification and electron microscopy analysis</td>
</tr>
</tbody>
</table>

### Challenges ahead

Though NGS technology has the potential to transform many aspects of oncology diagnosis, treatments and clinical trials, there are several important challenges and ethical considerations.

**Drivers versus passengers:** Distinguishing benign passenger mutations from those relevant to pathogenesis (ie, driver mutations) is one of the biggest challenges in cancer genomic discovery. Since many cancer genes contribute to cancer development in only a small fraction of tumors (for example, only approximately 10% of colorectal cancers have a BRAF mutation), large sample sets must be analyzed and hundreds of samples need to be sequenced to distinguish infrequently mutated cancer genes from genes with random clusters of passenger mutations. Optimal bioinformatics for NGS tumor analysis needs to incorporate NGS genomic data from high frequency pathways, hotspots, cancer genomic databases, and transcriptome and epigenome data if available. While there are a growing number of functional prediction databases (such as DriverDB) that incorporate NGS exome data, annotation databases, and bioinformatics algorithms, these resources are still limited and not ready for utilization for clinical trial selection.

**Exome versus whole-genome sequencing:** Owing to financial constraints and ease of interpretation, sequencing exomes (protein-coding genomic regions, which constitute approximately 1% of the whole genome) rather than whole genomes is still debatable and needs to be systematically investigated.

**Data challenges:** NGS and the resulting rapid increase in genome-scale data production have created great challenges in data integration. The major hurdles to integration among multiple samples and techniques are heterogeneity of the experimental and analytic protocols, varying levels of data quality, and differences in data representation. Data integration will remain challenging as more cancer genomes are sequenced, and innovative bioinformatics strategies are needed to facilitate this process.
In cancer genomics, since hundreds to thousands of genes need to be scrutinized in studies to identify molecular biomarkers in selected cancers, the huge data produced using NGS present a challenge in identifying true positives in the midst of large number of statistical hypotheses and comparisons.

**Regulatory implications:** To make therapeutic decisions for large numbers of exploratory biomarkers that would be studied during clinical trials, NGS assays should be done in a CLIA certified environment. Further, in order to identify and validate biomarkers and targeted therapeutics, awareness and education of new clinical trial designs among regulatory authorities will ensure that effective therapies reach patients efficiently without compromising their safety.

**References**


**Onikepe (Onyx) Adegbola, MD PhD**, Global Head, Scientific Affairs, Quest Diagnostics Clinical Trials

Dr Adegbola is currently responsible for leading the Scientific Affairs function at Quest Diagnostics Clinical Trials. In this role, she ensures the Scientific Affairs function leverages the scientific expertise and assay capabilities within all of Quest Diagnostics for clinical trial sponsors.

Dr Adegbola has 15 years of biomarker, translational medicine, clinical trial, and commercialization experience in the lab, pharma and medical device space. Prior to joining Quest she held roles at Novartis, Bayer Pharma and GE Healthcare. She received her M.D. from the University of Lagos, Nigeria and was awarded her Ph.D from The John Hopkins University of Medicine. She received pathology residency training at Columbia University and trained in yeast genetics at UCSF. She also completed a residency in Nuclear Medicine at the University of Pennsylvania.
Quest Diagnostics Advanced Sequencing Core Laboratory

Jamie Platt, PhD and Heather Sanders, PhD

Quest Diagnostics Advanced Sequencing Core Laboratory is a state-of-the-art facility intent on acquiring the latest technologies and platforms. We have nearly a decade of experience developing clinical assays on NGS platforms. Highlights of the clinical assays demonstrating our advanced capabilities include the following:

- **HIV-1 Genotypic Coreceptor Tropism with Reflex to Ultradeep Sequencing**
  - The first HIV Tropism assay to employ next generation ultra-deep sequencing to select antiretroviral drug regimen for treating HIV-1 patients released in 2008
  - Clinical trials utilizing this assay led to revised guidelines including ultra-deep sequencing for treatment selection in HIV patients

- **BRCAvantage single site, comprehensive, and Ashkenazi Jewish Screen**

- **OncoVantageSolid Tumor Mutation Profiling**
  - Mutation profiling of 34 genes with clinical implications in multiple solid tumor types.
  - Content is broad but focused on clinically actionable mutations
  - Enhanced reporting provides the client with meaningful results, including clinical implications of each mutation identified when applicable

Further endeavors of the Advanced Sequencing core laboratory include more comprehensive cancer and inherited disease assays, whole exome sequencing (including tumor/normal pairs), copy number variation profiling, transcriptome sequencing, circulating tumor nucleic acid detection, and epigenetics among others. The Advanced Sequencing core laboratory possesses high sequencing capacity and data management and storage capabilities. Among the ever-growing newest technologies, current instrumentation includes multiple MiSeq, HiSeq2500, Ion PGM, Ion Proton, GS Junior, and GS Flex sequencing platforms. By maintaining our experience with the most current advanced sequencing technologies, Quest Diagnostics aims to provide the most sophisticated and cost effective testing solutions.

---

**Jamie L. Platt, Ph.D., CGMBS, MB(ASCP)**, Scientific Director, Advanced Sequencing, Quest Diagnostics Nichols Institute

Dr. Platt received her Ph.D. in Molecular & Cellular Biology from Oregon State University and completed postdoctoral training in population genetics at the University of California, Berkeley. She has extensive expertise in sequencing and molecular systematics, including population genetics. Jamie has over 12 years of experience in clinical molecular diagnostics which she has used to bring NGS-based tests into the clinical lab. During her 12 years at Quest Diagnostics, Jamie has led the effort to develop and validated numerous sequencing-based...
tests in infectious diseases, oncology and genetics. For the past decade, she has focused her scientific efforts specifically on NGS and other advanced sequencing technologies, in an effort to improve the information available to researchers, physicians, and ultimately patients.

Heather R. Sanders, Ph.D, Principal Scientist, Oncology R&D, Quest Diagnostics Nichols Institute
Dr. Sanders received her Ph.D. degree in Microbiology at the University of California, Riverside where she furthered her studies as a postdoctoral researcher. She has been developing molecular diagnostic assays for Quest Diagnostics since 2006. Her work in the Oncology department has resulted in five patent filings for which she is named as Senior Inventor as well as authorship of several scientific publications. She has recently expanded her role to development of molecular diagnostic assays spanning multiple disciplines as Sr. Staff Scientist of Advanced Sequencing. Dr. Sanders is currently ASCP certified as a Molecular Biology Technologist and serves as on the editorial board for Molecular Cytogenetics.

Latest News
UCSF, Quest Diagnostics Alliance Aims to Advance Precision Medicine
Quest Diagnostics and the University of California, San Francisco (UCSF) have joined forces to translate precision medicine research into clinical diagnostics. With a special focus on autism, oncology, neurology, and women’s health, this partnership will combine the discoveries and research capabilities of UCSF with Quest Diagnostic’s expertise in data pooling and analysis. The collaboration launches with two projects already underway. One aims to identify genetic mutations associated with autism to aid in its diagnosis, while the second focuses on identifying biomarkers to guide the treatment of glioma brain tumors. As a resource, scientists from both organizations will use laboratory-based diagnostics, imaging procedures, and population analysis based on Quest Diagnostic’s national Health Trends database, which is the largest private clinical database in the U.S. Quest Diagnostics will then independently develop and validate any lab-developed tests for clinical use that emerge from the collaboration’s research.

As conveyed by Dr. Jay Wohlgemuth, MD, senior vice president of science and innovation at Quest Diagnostics; “Advances in technology and science have identified many promising opportunities to improve outcomes through insights revealed by novel diagnostic solutions, yet fulfilling the full potential of these opportunities often hinges on translational clinical studies which validate their value”. This unique collaboration between UCSF and Quest Diagnostics brings together the finest researchers and
clinicians in the country to accelerate the development of a product pipeline of scientific discoveries as clinically valuable diagnostic solutions that enable precision medicine for improved outcomes.”

**Quest Diagnostics NGS-based Dx partnership with Life Tech and Illumina Sequencing Systems**

Quest Diagnostics has struck multi-year agreements with both Illumina and Life Technologies to use their respective next-generation sequencing technologies for clinical laboratory testing. Under two separate agreements, Quest Diagnostics will have the right to develop and offer molecular laboratory-developed tests using Illumina’s MiSeq and Life Tech’s Ion Torrent platforms and the respective related reagents for clinicians and clinical trials performed by its pharmaceutical and biotechnology clients. The agreements will enable Quest Diagnostics to “build on our successes in NGS to accelerate the clinical benefit and positive impact of this technology for patients and providers across several disease areas, including cancer, neurology and women’s health. Last year, Quest Diagnostics began offering its next-gen sequencing-based BRCAvantage to assess mutations in the BRCA1 and BRCA2 genes related to hereditary breast cancer. It also offers next-gen sequencing testing services to aid in the diagnosis of myelodysplastic syndromes. Two years ago, Quest Diagnostics began offering an HIV tropism test based on next-gen sequencing and at the time, the company said it was evaluating all the desktop sequencing instruments — the Ion Torrent, MiSeq, and Roche’s 454 GS Junior — for future NGS-based diagnostics.

**Oncovantage test offering**

Over the past decade, cancer has become the largest therapeutic focus with the most significant increase in new drug development. From 2001 to 2010, the number of new cancer drug projects from phase I Clinical Trials through submission has tripled (Arrowsmith 2012). Along with these efforts, it has also become prudent to define biomarkers that aid in the selection of patients for treatment. In fact, this has been a major focus of related Draft Guidances and Concept papers through the FDA (Kelloff and Sigman 2012). The heterogeneity of solid tumors has broadened the scope of cell signaling pathways that are targeted by these new therapeutics. With this increase in targeted pathways and signaling molecules, the need for broader molecular profiling of gene disruption is necessary to apply this degree of personalized medicine.

Quest Diagnostics “OncoVantage” test encompasses somatic mutation detection in all genes that are currently routinely tested in solid tumors. These include EGFR (NSCLC), KRAS (NSCLC and CRC), BRAF (melanoma and thyroid cancer), HRAS (thyroid cancer), NRAS (CRC, melanoma and thyroid cancer), KIT (GIST and melanoma), PDGFRa (GIST), PIK3CA (CRC), and TP53 (multiple tumor types). Not only are all of these genes sequenced by OncoVantage, but an additional 25 genes with current or potential clinical relevance are included at a cost similar to just a few standard sequencing tests.

The benefit of including these additional 25 genes was represented in the results from the Cancer Genome Atlas (TCGA). TCGA is a coordinated study between the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI) that has involved whole exome sequencing (among other technologies) of hundreds of tumor specimens for a variety of cancer types. Reviewing the results from this study revealed that many mutations occur in genes for which routine ancillary testing is not performed for particular solid tumor types. For example, according to TCGA provisional data, 58% of
lung adenocarcinomas (n=229), 40% of colorectal tumors (n=585), 70% of melanomas (n=228), and 51% breast cancer (n=507; TCGA, Nature 2012) tumors harbored mutations in genes (excluding TP53) from our 34-gene panel that are not routinely tested for in the clinical laboratory. More than 78% (26/33) of these genes have, at minimum, pre-clinical evidence for an association with sensitivity or resistance to specific cancer treatments. Based on these results, there is clearly a significant benefit to interrogating these additional genes comprising the OncoVantage assay.

**Featured Case Study:**

**Innovative Testing Solutions for Patient Stratification in Relation to Lung Cancer Trial Recruitment**

**Background**
Detection of ALK gene rearrangement at chromosome 2p23 in patients with lung adenocarcinoma defines eligibility for targeted therapy (i.e., crizotinib) often leading to an excellent clinical response.

Fluorescence in situ hybridization (FISH) targeting the ALK gene utilizes the FDA approved Vysis ALK Break Apart FISH probe, a dual color DNA probe encompassing the 5’ and 3’ ends of the ALK gene. In a negative case, the dual colors would be represented as fused signals indicating an intact ALK gene (Figure 1A). In a clear positive case for ALK rearrangement, the FISH assay should show a separation of the dual color signals (Figure 1B), or, alternatively, a clear deletion of the green labeled portion of the probe set. Variant signal patterns, however, are frequently detected contributing to false positive, false negative and or equivocal findings.

We report one case that showed a diminished signal intensity of the probe component labeled in green in a large proportion of the cells examined (Figure 1C). The question arose whether this finding represented a rare ALK rearrangement or not.

**Comprehensive Testing Solution**
An RT-PCR based molecular assay was validated to detect increased ALK transcription in cases positive for ALK rearrangement. Thus, the cited case was subjected to this supplemental molecular study. The result was negative (see Figure 1D). Only the assay endogenous control was detected, but not ALK. This solved the uncertainty of the rare finding by FISH. Thus, the RT-PCR ALK rearrangement assay is necessary to further evaluate cases with FISH variant signal patterns that are encountered in clinical practice. The judicious use of these combined tools avoids false positive or false negative results, alleviating the uncertainty of variant signal patterns that occur in some FISH assays.

Hence, central laboratories supporting Oncology clinical trial testing should be prepared to address FISH ALK signal variations with a robustly validated RT-PCR assay for ALK rearrangements.

Reference: Dai et al., Mol Cytogenet. 2012 Dec 3;5(1):44. doi: 10.1186/1755-8166-5-44 (Figure 1A - 1.D)
Figure 1. Fluorescent images of cells from formalin fixed paraffin embedded lung tissue hybridized with Vysis ALK Break Apart probe (Abbott Molecular, Abbott Park, IL).

Figure 1.A Normal patient sample showing the signal pattern of 2 fusion (2F), indicating negative for rearrangement of ALK gene.

Figure 1.B An abnormal sample with signal pattern of 1O1G1F, positive for rearrangement of ALK gene.

Figure 1.C Case with variant signal patterns such as 1O1G(dim)1F

Figure 1.D Subsequent RT-PCR profile of the Fig. 1.C case showing no additional transcripts of ALK gene detected.
Recent Publications

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

Screening for Cervical Cancer in Low-Resource Settings in 2011. Barbara Patrizzi, CT, ASCP
Cytology, Quest Diagnostics Inc, Horsham, PA 19044

Isochromosome Yp and jumping translocation of Yq resulting in five cell lines in an infertile male: a case report and review of the literature. Hemmat M, Hemmat O, Boyar FZ. Cytogenetics Department, Quest Diagnostics Nichols Institute, 33608 Ortega Highway, San Juan Capistrano, CA 92690, USA.

A National Assessment of Warfarin Anticoagulation Therapy for Stroke Prevention in Atrial Fibrillation
Jeffrey S. Dlott; Roberta A. George; Xiaohua Huang; Mouneer Odeh; Harvey W. Kaufman et al.
Quest Diagnostics Nichols Institute, Chantilly, VA; Quest Diagnostics, West Norriton, PA; Quest Diagnostics, Madison, NJ

Wang Y: Cancer Diagnostics Service, Quest Diagnostics Nichols Institute, Chantilly, VA 20151, United States.

Clarke NJ: Quest Diagnostics Nichols Institute, San Juan Capistrano, California 92675.

Serological assessment of samples from patients complaining of dyspepsia.
Mortlock, S. Quest Diagnostics Clinical Trials, Heston, Middlesex, UK.

Unintended reporting of misleading hb A(1c) values when using assays incapable of detecting hemoglobin variants.
Rhea JM, Koch D, Ritchie J, Singh HV, Young AN, Burgess T, Molinaro RJ.
Burgess, T: Laboratory Medicine, Quest Diagnostics, Atlanta, Georgia

Measurement of estradiol-challenges ahead.
Stanczyk FZ, Clarke NJ.
Clarke, NJ. Steroids Department (N.J.C.), Quest Diagnostics Nichols Institute, San Juan Capistrano, California 92675.

Deletions of the PRKAR1A Locus at 17q24.2-q24.3 in Carney Complex: Genotype-Phenotype Correlations and Implications for Genetic Testing.
New Assays at Quest Diagnostics

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

1. Lung Cancer (NSCLC), ROS1 (6q22) Rearrangement
ROS1 rearrangement has been identified in 1.7% of non-small-cell lung cancer (NSCLC) by using a FISH assay. Treatment with ALK/MET tyrosine kinase inhibitors such as crizotinib (Xalkori(R)) has shown early evidence of therapeutic efficacy in ROS1-rearranged NSCLC (Bergethon, et al. J Clin Oncol. 2012;30:863-870).

   Method: Fluorescent in-situ hybridisation

2. MYD88, Mutation Analysis
L265P mutation in the MYD88 gene is found in approximately 90% of Waldenström macroglobulinemia and IgM-expressing lymphoplasmacytic lymphoma (LPL). There is a low incidence of L265P MYD88 mutation in other systemic CD5-negative B-cell lymphoproliferative disorders including atypical chronic lymphocytic leukemia, nodal marginal zone lymphoma (MZL), splenic MZL and mucosa-associated lymphoid tissue (MALT)-type MZL. This assay sensitively detects the L265P MYD88 mutation and can be used to help diagnose Waldenström macroglobulinemia or IgM-expressing lymphoplasmacytic lymphoma and to help in stratifying or subclassifying patients with IgM monoclonal gammopathy.

   Method: PCR-based DNA pyrosequencing

3. KIT D816, Mutation Analysis (Mastocytosis)
Point mutation of the KIT oncogene at codon 816 (D816V) is seen in >90% of systemic mastocytosis (SM) cases. The presence of KIT D816V mutation is one of the minor criteria for diagnosis of SM and mutation testing can assist in diagnosis, particularly in limited specimens. KIT D816 mutations, including D816V, D816H and D816Y, are also the most common KIT mutations seen in the core-binding factor acute myeloid leukemia (AML). In both t(8;21) and inv(16)/t(16;16) AML, cases with KIT D816 mutation are associated with worse outcomes than unmutated cases. This assay sensitively detects the KIT D816V mutation down to 2%. If KIT mutation testing of gastrointestinal stromal tumor (GIST) or melanoma is needed, test code 19961 should be used instead, which tests for exons 8, 9, 11,
13 and 17 of the KIT gene by Sanger sequencing.

**Method:** PCR-based DNA pyrosequencing

4. **Multiplex Cytokines: MSD 10-v-Plex Pro-Inflammatory Panel**

Multiplexing analytes for testing in a lab has been a challenge while performing long term clinical trial studies due to lack of inter-lot bridging at the vendor level along with short shelf life of reagents. Recently, Meso Scale Discovery (MSD), with guidance from pharma and diagnostic laboratory customers like QDCT, has developed V-plex multiplex assay kits which seem to address these challenges. The V-plex kits which expire thirty months after manufacturing and include Quality Controls have been validated by MSD to NCCLS and FDA Guidance Documents.

The reference calibrators for these V-plex assays are anchored to NIBSC/WHO standards which will help us provide ‘Gold Standard’ analyte absolute values to clients for their studies.

Our Biomarker Lab in Valencia, CA, were the first to collaborate with MSD to validate their 10-V-Plex Pro-Inflammatory Panel-1 consisting of IL1beta; IL-2; IL-4; IL-6; IL-8; IL-10; IL-12p70; IL-13; TNF-alpha and IFN-gamma

Method: electrochemiluminescence detection to detect binding events on patterned arrays.

5. **QuestAssureD™ for Infants, 25-Hydroxyvitamin D**

This assay employs advanced liquid chromatography - tandem mass spectrometry, allowing accurate measurement of Vitamin D in the presence of c3-epimer. While this assay will produce accurate Vitamin D results on patients of any age, it is specifically indicated for infants (0 - 2 years of age).

**Method:** LCMSMS

6. **Prenat-IQ™, Noninvasive Prenatal Test (NIPT)**

Fetal chromosomal aneuploidies for chromosomes 13, 18, 21 and X and Y, as well as triploidy, will be determined through this non-invasive test using plasma obtained from the mother’s whole blood.

**Method:** SNP targeted sequencing

7. **Cardio IQ™ ST2 (Soluble) and proBNP (N-Terminal)**

A panel that contains both ST2 and NT-proBNP in conjunction with clinical factors can help physicians more effectively identify individuals at risk of HF progression than the individual tests and therefore can help physicians select appropriate therapeutic regimens.

**Method:** Enzyme-linked immunosorbant assay

8. **Lipoprotein Fractionation, Ion Mobility**

Ion Mobility offers the only direct measurement of lipoprotein particle size and concentration for each lipoprotein from HDL3 to large VLDL.

**Method:** IM works on the principle that particles of a given size and charge state behave in a predictable manner, when carried in a laminar-air flow through an electric field. Using this technology, particles are separate based on size in a Dynamic Mobility Analyzer (DMA). Collected particles at each size are then counted in a condensation particle counter. In this way the actual number of particles of any given size can be determined and converted to a concentration of particles.

9. **Dabigatran with Reflex to Thrombin Time**

Dabigatran is a new oral direct anti-thrombin drug and an alternative to Warfarin. The drug received FDA clearance in 2010 for the indication of stroke prevention in patients with atrial fibrillation. Routinely, the drug does not need to be monitored, however, there are instances where monitoring is needed: (1) Determination of failure of therapy vs. poor compliance (2) Potential dose adjustment required for renal or hepatic dysfunction (3) Titration.

**Method:** Clotting assay
10. Antigen-induced Lymphocyte Proliferation Panel (Candida, Tetanus, TB PPD) Measurement of human lymphocyte proliferative responses to various stimuli is a fundamental technique used to assess their biological status and functions. Lymphocyte proliferation response to antigens, such as Candida, tetanus toxoid and tuberculin purified protein derivative (PPD), are evaluated as a function of memory in cell-mediated immunity.

Method: Cell culture; scintillation counter

11. Mitogen-induced Lymphocyte Proliferation Panel (PHA, Con A, PWM) Includes Phytohemagglutinin (PHA)-induced Lymphocyte Proliferation; Concanavalin A (Con A)-induced Lymphocyte Proliferation; Pokeweed Mitogen (PWM) induced Lymphocyte Proliferation.

Mitogens, such as plant lectins phytohemagglutinin (PHA), concanavalin A (Con A) and pokeweed mitogen (PWM), are able to nonspecifically stimulate lymphocyte proliferation and used to evaluate patient immune responsiveness.

Method: Cell culture; scintillation counter

12. Cytomegalovirus (CMV) genotype Mutations in specific codons of the CMV UL97 gene are associated with resistance to ganciclovir while mutations in specific codons of the CMV UL54 gene are associated with resistance to ganciclovir, foscarnet and/or cidofovir. Mutations can consist of changes in codons encoding the amino acids as well as deletions of nucleotides.

Method: Detection of these mutations is based upon PCR amplification of genomic CMV DNA followed by DNA sequencing.

13. Anti-titin autoantibodies. Autoantibodies to titin are closely associated with late-onset myasthenia gravis (MG), and are detected in significant numbers of patients with thymoma. As anti-titin correlates with MG severity, antibody status may be useful in assessing prognosis, treatment and followup, particularly in initiating more aggressive treatment.

The absence of titin (and RyR) antibodies strongly excludes thymoma.

Method: This test employs a standard microplate based immunoassay procedure, where the titin antigen (MGT-30) is coated directly into the plate's wells.
For more information, please call:
North America: 800.209.9816
London, UK: +44.208.377.3300
Or, email Clinical.Trials@QuestDiagnostics.com
Or, visit questdiagnostics.com/clinicaltrials