A new era in molecular diagnostics: The TRUGENE® DNA Sequencing System

The OpenGene® DNA Sequencing System, the platform used with TRUGENE products, is a powerful genetic analysis platform developed for the more effective management of treatable diseases. Currently integrating the Siemens TRUGENE HIV-1 Genotyping Kit, which has emerged as a key component to individualized patient management and clinical laboratory referencing, it has prepared the path for further pioneering kits.

By Rob M. Lloyd, Jr.
Overview of molecular genetics and diagnostics

The use of molecular diagnostics testing platforms that use either genomic or proteomic technologies have increased dramatically. Currently the fastest-growing business segment in clinical diagnostics, clinicians find the tests highly valuable in helping them select appropriate treatment strategies. These new genomic and proteomic technologies generally include analysis of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or protein-specific targets to predict a disease or predisposition of an individual to a disease (personalized genetic medicine). Before development of molecular diagnostic tests, clinicians were limited to less sensitive assays that did not capture either host or infectious pathogen genetic information.

Today, a variety of molecular genetic diagnostic tests are used for routine patient management. The analysis of specific gene sequences provides important diagnostic information for identifying a pathogenic agent, predicting disease, monitoring disease, choosing treatment options, and subsequently, for determining the effectiveness of the specified therapy or treatment. Additionally, novel molecular technologies with diagnostic applications have become an important tool in drug discovery and preclinical drug development.

Resistence testing in HIV-1 disease management

HIV drug-resistance testing has become an integral part of patient care and helps providers choose antiretroviral drugs more wisely. These tests identify drugs that may be less active for their patient based on appearance of drug-resistance mutations known to reduce susceptibility to specific drugs. There are two parts to the TRUGENE Resistance test: determination of the appearance of drug-resistance mutations in the sequence of the virus, and an interpretation of the virus sequence, with report of which drugs have diminished activity (possible resistance) or full resistance. Although there are many different ways to obtain the sequence, it is the interpretation of the resistance mutation pattern identified through sequencing that is the key for resistance testing. TRUGENE uses an expert committee, composed of 10 leaders in the HIV drug resistance field, who meet annually to review published literature and the work presented in national and international conferences in order to keep the algorithm up to date. There is a validation process and changes are submitted for FDA approval and change in the TRUGENE package insert. This test was previously validated in prospective clinical trials where it was shown that recognition of resistance mutations that indicate diminished susceptibility to specific drugs helps clinicians achieve greater rates of virologic suppression in patients who had TRUGENE Resistance testing than in patients in whom drugs were selected without the benefit of resistance testing.

The recommendation to perform baseline testing, before starting the initial antiretroviral regimen, is more recent and was previously only suggested. The most popular initial regimens rely on activity from the non-nucleoside reverse transcriptase inhibitors, but resistance to this class is seen in over 10 percent of individuals during primary infection and rates of non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance do not appreciably diminish over a period of several years. This is a critical difference between resistance that emerges under selective pressure of therapy and resistance that is conferred during primary infection. When only wild type virus...
(without any resistance mutations) is transmitted during primary infection, the reservoirs in latently-infected cells reflect this lack of resistance. In this case, resistance emerges under selective pressure of suboptimal therapy. If antiretroviral therapy is withdrawn, the circulating virus population emerges from reservoirs in cells that largely lack the resistance mutations. There still is memory of the resistance from new proviral DNA formation during initial emergence of drug resistance, and the resistant populations rapidly emerge if selective pressure is reapplied. The situation is different when the latent reservoir is largely comprised of drug-resistant virus transmitted during acute infection. In this case, even without selective pressure of antiretroviral drugs, viruses with the drug-resistant mutations persist, since this is the virus that comprises the reservoir. It is recognition of this phenomenon that has led to strong recommendations to perform a resistance test prior to starting therapy, no matter how long it has been since primary infection. This recommendation is supported by a recent case control study to assess the effect of baseline drug resistance on response to efavirenz-containing regimens in ACTG 5095. There were 57 case patients (with virologic failure) and 163 control patients (without virologic failure) randomly selected, with 136 additional patients added with virologic failure to make up a case-cohort sample of 356 (193 with virologic failure and 163 with virologic failure). The prevalence of NNRTI resistance was 5 percent overall, but the risk of failure was more than doubled by the presence of NNRTI resistance mutations at baseline. These were present in 8 percent of subjects with virologic failure and 2 percent of subjects without virologic failure. All of the patients with NNRTI resistance mutations at baseline failed therapy eventually, reinforcing the importance of baseline resistance testing to make sure appropriate initial regimens are selected. If there are NNRTI resistance mutations seen at baseline, ritonavir-boosted protease inhibitor-based therapy should be used instead for the initial regimen in order to increase the potential for long-term response. This is the best way to conserve active drugs, since NNRTI failure is commonly associated with resistance to nucleoside reverse transcriptase inhibitors as well. The US Department of Health and Human Services (DHHS) guidelines for HIV drug resistance testing are shown as they appear in the Guidelines document, in Figure 1.

Hepatitis C Virus

The Hepatitis C Virus (HCV) is blood-borne and can cause progressive liver disease. HCV leads the world as the most common chronic blood borne infection in the world and is a leading cause of chronic liver disease, including cirrhosis. HCV infection is a major health concern with an estimated three percent of the world’s population infected with the virus and more than 170 million persons at risk of fulminant hepatitis disease. HCV is a small-enveloped, single-stranded RNA virus with approximately 9,600 nucleotides per genome. As with many RNA viruses, HCV has a high genetic mutation rate and because of this, distinct types of HCV and more than 100 subtypes have been identified by genetic sequencing technology. Accurate diagnostic testing of a specified “TYPE” of HCV infection is important for predicting the potential outcome or efficacy of current treatment.
HCV genotyping

HCV "typing" and accurate sub-typing have become increasingly relevant to clinical development of new drugs and subsequent treatment strategies. Response to therapy with interferon and ribavirin (the current recommended treatment) is worse with HCV "TYPE" 1 (the most common type), as compared with that seen with HCV genotypes 2 and 3. HCV TYPE determination is routinely performed in clinical trials and is increasingly the standard for patient management for current treatment strategies.

Hepatitis B Virus

The Hepatitis B Virus (HBV) is a major causative agent for chronic hepatitis, cirrhosis, and is the leading cause of chronic liver disease and hepatocellular carcinoma worldwide. An estimated 350 million individuals are infected with HBV despite the availability of an effective preventative vaccine. Although vaccination programs have reduced the incidence of new HBV infections, the virus remains a major public health problem in parts of Asia and Africa. Despite effective drug treatment, greater than 500,000 worldwide deaths per year are associated with HBV infection. Development of therapies, which include nucleoside and nucleotide analogs shown to be safe and effective drugs for HBV, have been developed and approved for use. These advances in drug therapy are complicated with drug resistance and new guidelines for the treatment of chronic HBV have been published. The viral genome is highly polymorphic due to an error-prone reverse transcription enzyme and subtype variants are abundant. The successful treatment of chronic HBV may ultimately follow a combination therapy approach similar to HIV-1 that will also rely on molecular diagnostic approaches to formulate the most effective therapy.

HBV genotype testing

Several significant variations in geographical distribution of HBV genotypes have been identified worldwide and eight characterized "types" of human HBV (designated A–H) have been reported. Some of these "types" could have varying clinical response to new drugs in development and clinical studies are ongoing to determine "type" specific treatment outcomes.

The automated "typing" module of the TRUGENE HBV Genotyping Kit utilizes Surface Antigen (SA) gene sequence and best fit phylogenetic analysis using a comprehensive genetic library of validated HBV reference sequences. The second portion of this assay's automated analysis utilizes the polymerase gene region for nucleotide detection of mutations that are associated with drug resistance.

Drug-resistance in HBV is well documented in the literature, and the need for early detection of emerging mutations in various motifs of the polymerase gene is clinically relevant. HBV Genotyping allows for current clinical applications for drug-resistant mutations to FDA-approved, clinically-relevant drugs and drugs in discovery phase. The use of diagnostic resistance testing is limited by the validation and test incorporation of reported resistance-associated mutations. These tests can be updated to include new drugs as they become available.

OpenGene "molecular diagnostic" DNA sequencing platform

OpenGene is the generic name for one of the world’s most recognized molecular diagnostic DNA sequencing platforms. The complete OpenGene DNA Sequencing System contains a fully-integrated, FDA-approved genetic analysis platform, a series of comprehensive software suites including automated sequence analysis, editing and reporting tools, and the TRUGENE HIV-1 Genotyping Kit. This represents the first FDA-cleared molecular diagnostic sequencing platform (September 2001). The OpenGene System currently focuses on pathogenic or infectious disease agents such as HIV-1, but can be easily adapted to a broader array of personalized genetic diagnostic testing applications. The TRUGENE System currently integrates an FDA-approved HIV-1 Drug Resistance Genotyping Kit (covering Protease and Reverse Transcriptase genes), a genetic sequencing platform (Tower® Instrument) utilizing high-speed, thin-slab electrophoresis technology, and an automated genetic reporting algorithm that automatically generates an FDA-cleared "Resistance Report".

DHHS recommendations for HIV drug-resistance testing

Strength of recommendation: A, strong; B, moderate; C, optional; D, should usually not be offered; E, should never be offered; Quality of evidence for recommendation: I, at least one randomized trial; II, clinical trials with laboratory results; III, expert opinion.

DHHS, US Department of Health and Human Services