The rise of multiplexing in the molecular laboratory

By Martin Crockard, PhD

The completion of the mapping of the human genome more than a decade ago has catalyzed a dramatic shift in approaches to diagnostics. Today we stand on the threshold of the "Genomic Era," in a time when genetic testing and pharmacogenomics have become mainstays in the diagnosis and treatment of disease. From genotyping patients, to predicting drug response, to stratifying patients according to the risk of a disease, molecular testing has become mainstream, and the promise of personalized medicine has become the norm.

There are now more than 15,000 tests for more than 2,800 genes. The molecular diagnostics market has become the fastest growing segment of the IVD industry, with assays serving multiple disease areas and breaking new boundaries in personalized healthcare.

With the emphasis on personalization of treatment, recent years have witnessed pharmaceutical companies shifting their pipeline strategy from low-value, “one-size-fits-all” therapies to high-value, targeted therapies applicable to genetically defined populations. Within the genetic laboratory setting, the emergence of highly targeted multiplex molecular diagnostic assays has enabled healthcare systems to provide personalized medicine in a cost-effective and clinically relevant way.

The case for multiplex assays

The benefits of adopting multiplex assays can be seen particularly in the area of infectious disease, where detection of co-infections is vital in understanding the mechanism of infection. In a recent paper by Memish et al, published in June 2014, a study into the etiology of severe community-acquired pneumonia (CAP) during the 2013 Hajj highlighted the presence of co-infections in pilgrims diagnosed with severe CAP and admitted to a healthcare facility. The study found that co-infections were present in 68.4% of patients, with 80.7% of patients harboring more than one respiratory pathogen.

Both bacterial and viral infections were present in 65.3% of patients, demonstrating the complexity of respiratory infections and emphasising the need for further investigation to accurately detect all pathogens present in order to better measure and manage infection. This is particularly necessary in respiratory and sexually transmitted infections (STIs), where the inappropriate use of antibiotics can prolong infection exposure and add to the burden of antibiotic resistance currently threatening public health worldwide.

However, despite the availability of powerful molecular multiplex diagnostic assays across a range of conditions, the use of such tests in the area of inherited disease is far from routine. Many “traditional” diagnostic tests continue to under-diagnose, leading to missed opportunities for early and appropriate therapy intervention for potentially life-threatening diseases. One prime example in which a multiplex molecular diagnostic approach can improve health is testing for familial hypercholesterolemia (FH).

Familial hypercholesterolemia

FH is an inherited genetic disorder, affecting the body’s ability to clear LDL-cholesterol (LDL-C), which, if untreated, can lead typically to atherosclerosis and premature onset of cardiovascular disease in men in their 40s and women in their 50s. The main gene responsible for the genetic defects associated with FH is the low-density lipoprotein receptor (LDLR) gene. At least one in 500 people in the general population harbors mutations in this gene.

While FH can be diagnosed clinically or genetically, it is recommended by UK, U.S., and international guidelines that probable or possible FH patients undergo a DNA test to confirm the diagnosis of FH. Recommendations also advocate that once an activating mutation has been found in one family member (the index case), cascade screening of that mutation in first-degree relatives of the index case should be conducted to further assist in case identification and improve patient outcomes.

Diagnosis of FH through lipid profiling (LDL, HDL, and total cholesterol, for example) is not sufficient to distinguish FH from other hyperlipidemias and will not guide appropriate treatment. FH requires a much more aggressive treatment regime, with higher levels of statins or combined therapies to effectively reduce the risk of CHD and stroke, so definitive mutation analysis is a more accurate option.

Further, FH management can be dictated by the type of mutation harbored by the patient, such as the poorer response to lipid-lowering therapy observed with specific LDLR mutations. Consequently, accurately identifying the gene mutation involved can potentially help the clinician decide how aggressive the treatment strategy should be. Genetic diagnosis of FH through adoption of routine screening will allow patients to receive appropriate therapeutic and interventional treatment to reduce morbidity and mortality associated with cardiovascular disease.

Pioneering multiplex diagnostic assays, tailored to incorporate the relevant FH-causing mutations, provide a promising future for both the genetic laboratories, where a rapid, cost-effective approach to determine mutational status in cases of suspected FH is enabled, and the patient, whose treatment and care pathway is managed effectively.

Determining mutational status

To date, more than 1,200 different LDLR mutations have been reported, with prevalence varying among different populations. The abundance of different FH mutations can make genetic testing labor-intensive and costly, with many laboratories defaulting to performing expensive and lengthy next generation sequencing (NGS) tests in an effort to ensure a comprehensive mutational screen.

However, as our understanding of the genetic drivers of FH, as well as common population-specific mutations, increases, new novel multiplex assays are being developed to meet the continued on page 14

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clinical need and to screen for the most relevant mutations in FH. These assays delivering accurate results within hours rather than weeks at a significantly lower cost. 4

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References


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