MOLECULAR DIAGNOSTICS

NUCLEIC ACID TESTING APPLICATIONS

Molecular testing dominates labs, but human interface still necessary to interpret results

By Robin Hocevar

After decades of warning, lab automation has arrived and is here to stay. Though instrumentation has taken some of the human component out of the diagnostic equation, some argue that it’s more important than ever for laboratorians to be fluent in nucleic acid testing (NAT).

“Even though lab workers can now just put a tube in a magic machine that does all the work, it’s so important to know the science behind the magic,” explained Massimo Mangiola, PhD, director of special services, HLA, IRL, identity testing laboratory at Rhode Island Blood Center.

Even blood centers are moving to NAT to detect pathogens, he said. In microbiology, there’s been a tendency to switch to molecular testing for faster detection. Molecular testing also detects pathogens even when there are no clinical signs.

Yet, even the most sophisticated tests can’t interpret for all the possible co-occurring conditions that complicate laboratory testing. “No vendor can create an algorithm to cover every possible combination,” noted Mangiola. “You can’t completely rely on your instrument. Technical expertise is necessary for every test result because many results have a clinical impact in patient care.”

Categories of Amplification

Nucleic acid amplification falls into four subtypes: target amplification, probe amplification, signal amplification and whole genome sequencing. Within the four categories, virtually anything can be targeted.

“Choosing between the different categories comes down to the sensitivity of the assay and what you want to achieve,” stated Mangiola.

For sensitivity, using a Polymerase Chain Reaction (PCR) amplification may be the best, remarked Mangiola. This version encompasses endpoint PCR with specific primers or probes, real-time PCR (Q-PCR), nested PCR (with two sets of amplification primers, one internal to the other) and multiplex PCR (with two sets of amplification primers specific for different targets).

Endpoint PCR is most appropriate for Factor-V Leiden mutation, Prothrombin mutation and HLA disease associations while quantitative PCR is designed for CMV, EBV, BKV and STDs.

Government regulations dictate the testing direction for a laboratory.

“If you’re dealing with donor selection for transplantation or transfusion, there’s a lot of FDA regulation you need to follow,” he noted. “There are different regulations whether you’re monitoring patients, as the case may be post-transplantation monitoring of CMV. In this situation, you can use unregulated assays in agreement with the transplant center. However, with donors, you have to follow specific FDA regulations.”

Issues of Timing

Certainly, a sensitive method like timed PCR would be the better choice over, say, signal amplification when you want to detect a disease like CMV before any of the clinical warning signs.

In other situations, getting the fastest result takes the top priority. “Both tests are highly sensitive,” explained Mangiola. “The difference is in the primer and how fast you get the results. Real-time PCR is the fastest, with about 90 minutes from the blood test to the results. Endpoint PCR does amplification then something else before attaining results.”
Even more than speed, there’s increasing pressure on product manufacturers to create tests that can be replicated with almost perfect precision. “Demand has taken variability out of the mix,” confirmed Mangiola. “Even though you decide to use instrumentation, there can’t be any differences in timing or results when you’re monitoring the status of a disease or determining a viral load.

No discussion of PCR analysis would be complete without covering cost. If fast results and finances are both priorities, Mangiola mentioned the availability of SYBR Green chemistry. It’s the simplest and least costly approach to qPCR that utilizes DNA-binding fluorophores for non-specific detection of target DNA sequences. In this type of chemistry, the sensitivity and specificity of the assay is determined only by the quality of the primers.

SYBR Green is a naturally fluorescing dye that can intercalate between DNA bases. SYBR Green exhibits low fluorescence when unbound in solution, but starts to fluoresce brightly when associated with double-stranded DNA (dsDNA) and exposed to a suitable wavelength of light.

Whole Genome Sequencing

Though it’s an expensive option, there’s no questioning the domination of whole genome sequencing. The latest instruments are capable of producing hundreds of gigabases of valuable data in a single sequencing run and can detect virtually anything that can be studied from genomic DNA.

“Especially with whole genome sequencing, we’re closing the gaps on genes for which we previously didn’t know the precise sequence,” state Mangiola. “We’re learning the molecular approach is definitive and safe and there’s a huge push for everyone to get on board with molecular. It’s challenging for labs because this approach is very expensive.”

In general applications, it’s the job of the lab to determine the best testing methodology for the patient population. That’s the part of the job that Mangiola said will never be replaced by machinery and offers a unique opportunity for laboratory personnel to apply expertise.

“Nucleic acid testing is readily available and several companies can easily and cost-effectively create primers and detection chemistries for you,” he remarked. “But each lab needs to figure out which testing direction to go, based on the clinical need.”

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