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LEARNING OBJECTIVES

Upon completion of this article, the reader will be able to:

1. Recognize reasons for adding molecular testing to lab options.
2. Recognize current testing platforms used for real-time PCR.
3. Recognize advantages and disadvantages of different testing platforms.
4. Describe how patient population, workflow and staff experience/workload affect the choice of molecular platforms.

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Make the move to molecular diagnostics

By Elizabeth Palavecino, MD

With advances in instruments and assays, selecting a platform can be a difficult decision — but one with important ramifications for cost effectiveness and patient care. In recent years, sophisticated molecular-test methods for the diagnosis of infectious diseases have moved out of research and reference laboratories, and are now available for use in clinical microbiology laboratories in hospitals different sizes and different patient populations. In parallel with this transition, manufacturers have launched a wide variety of options — both instruments and assays — from which laboratories can choose. Molecular testing provides higher sensitivity and specificity than traditional methods. Making that first step into molecular testing can be vexing for both clinical lab directors and administrators. Selecting the right devices and assays is a complicated task with significant consequences for patient care and staff efficiency. The decision should be made only after key variables have been considered.

Moving toward in-house molecular testing is a complex decision, and it is made even more so by the scarcity of independent and reliable evaluations of assays and instruments.

Selecting a methodology

First, the lab needs to select from among several methodologies, each with unique features. Many clinical laboratories are drawn to real-time polymerase chain reaction (PCR) technology as compared to traditional PCR because the amplification and detection are done simultaneously in a closed system, thereby reducing the risk of contamination.

Another chief benefit of the technology is speed; like other molecular testing methodologies, real-time PCR can provide highly sensitive and specific results in a clinically relevant period of time. Not surprisingly, the number of devices using real-time PCR methodology has increased drastically and the features vary from manufacturer to manufacturer. Some offer more automation; some are more compact; some provide more flexibility in sample size and throughput rates.

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Selecting the right system

There are numerous suppliers of molecular-testing devices; and, again, each provides a set of advantages and disadvantages. Depending on the needs of a particular user, the main goal is to determine the test menu needed and then select the one or two instruments that can accommodate those needs.

Before discussing the options and criteria for selection, a note on terminology might be helpful. The U.S. Food and Drug Administration (FDA) reviews submissions for instruments and assays, and issues a clearance. In certain circumstances, as when there is an immediate need for certain kinds of tests to deal with serious health issues, the FDA may issue an emergency-use authorization (EUA). In 2009, for example, the FDA issued EUAs for a number of influenza H1N1 assays.

Assays that have not received FDA clearance or EUA can still provide useful information, but additional verification and validation procedures are required before

the facility can report the results of these assay-specific reagent (ASR) tests for clinical purposes. Consequently, a laboratory new to molecular testing might want to consider a system that offers at least some tests that have received FDA clearance or emergency-use authorization.

Making that first step into molecular testing ... can be vexing for both clinical lab directors and administrators.

It is beyond the scope of this article to provide a comprehensive overview of all the real-time PCR systems available for clinical laboratories. A few examples of such systems, however, are provided to illustrate the different levels of automation and the different assays that can be performed on each.

- 3M Integrated Cyclor (a collaboration between Focus Diagnostics, Cypress, CA, and 3M, Saint Paul, MN) is scalable from amplification and detection of preprocessed samples to integrated on-board sample preparation. The universal disk is able to process up to 96 samples simultaneously and employs a number of unique design features, including a proprietary adhesive to ensure sample integrity during processing. The FDA has issued an EUA for this system's Simplexa Influenza A/H1N1 (2009) test.
- The Cobas AmpliPrep/Cobas TaqMan 48 Analyzer from Roche, Basel, Switzerland, allows automated extraction, real-time amplification and detection of DNA or RNA for up to two simultaneous assays. Among other assays, its tests for quantification of HIV and hepatitis C virus have received FDA clearance.
- GeneXpert System from Cepheid, Sunnyvale, CA, integrates sample preparation, DNA amplification, and detection allowing single sample testing. Cepheid's Xpert assays that have received FDA clearance include tests for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile* (*C diff*), and vancomycin-resistant enterococcus (VRE), among others.
- SmartCycler System from Cepheid combines fast thermal ramp rates

and real-time detection. System capacity can be increased by connecting multiples of 16-module units. Among other assays, it can perform the FDA-cleared Cepheid Smart GBS test, a qualitative *in vitro* diagnostic test designed to detect Group B Streptococcus (GBS) DNA in vaginal/rectal specimens. Assays from other manufacturers can also be performed on this instrument, such as the BD GeneOhm MRSA assay from Becton, Dickinson and Company, Franklin Lakes, NJ, and ProFlu+ from Prodesse, Gen-Probe, San Diego, CA, all of which are FDA-cleared.

Additionally, there are technologies other than real-time PCR. For example, xTAG technology uses multiplex PCR — combined with the Luminex instrument from Luminex Molecular Diagnostics, Toronto, Canada, using a laser to discover the targets — to provide detection of multiple viral agents within a single specimen.

Some manufacturers have a limited test menu while others offer a wide range of tests for infectious-disease diagnosis and more. Indeed, the range of available assays that can be performed varies considerably from instrument to instrument. This means that clinical laboratories have to choose between limited capabilities (one instrument to run a few ID tests, for example) or a significant investment (in multiple and expensive instruments). This decision has implications for the lab's cost effectiveness and the institution's ability to provide relevant care.

As manufacturers strive to increase the functionality of their devices, they will also develop additional assays. They will also continue to improve the instruments, making them easier to use and more reliable. An example of this evolution is the recent attention to procedures for nucleic-acid extraction. In some devices today, this part of the testing process has been automated and connected to the automated amplification and detection steps. This has improved the reliability of results (by standardizing the procedure) and reduced turnaround time.

As a result of this automation and other improvements, some molecular tests may be considered suitable for

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“near-patient” use. Done correctly, near-patient testing can improve care and cost effectiveness, but caution should be taken with interpretation of the results. If personnel have insufficient experience with the procedure or with interpreting results, clinical liability may occur.

Key criteria

Each clinical laboratory will need to consider several parameters in order to decide on the device that best fits its needs. Some of these parameters are:

Patient population: The specific characteristics of the patients at one particular healthcare institution will define, in large part, the nature of the tests the lab will be undertaking and narrow the field of instruments to those that offer the appropriate assay. For example, if the institution has a large population of immunosuppressed patients, particularly organ- or stem-cell-transplant patients, the laboratory will want quantitative tests with a high degree of sensitivity and specificity to monitor infections with cytomegalovirus (CMV), BK virus, Epstein-Barr virus (EBV), and other viral agents. If the main goal is monitoring patients for hospital-acquired infections, the lab will want an instrument that offers tests for detection of MRSA or VRE.

Lab workflow and volume: Facilities that handle a high volume of tests for a particular infectious agent, for example, will want to look at an instrument that can accommodate batching of multiple samples; a lab that tends to perform lower volumes might want to consider an instrument that can handle single samples. And while it might sound like a secondary consideration, the footprint of the equipment is meaningful. In most labs, space is at a premium; a new system should not — and need not — crowd out or complicate the use of other important equipment.

Staff experience and workload: If the staff has limited experience or training, an

instrument with automated procedures for extracting and preparing samples may be preferable. Staff experience is especially critical for performing verification and validation for newly acquired instruments and tests. In the United States, all molecular tests, including FDA-cleared, modified, and laboratory-developed tests, require verification before implementation and reporting results.

The process of verification according to the Clinical Laboratory Improvement Amendments is intended to demonstrate performance specifications. For unmodified FDA-cleared tests, the lab must demonstrate it can obtain comparable results to those established by the manufacturer for accuracy, precision, and measuring range of results. For modified FDA-cleared test systems, in-house developed tests, or tests without provided performance specifications from the manufacturer, the lab must establish performance specifications for accuracy, precision, analytical sensitivity, analytical specificity, reportable range, and reference intervals. These are time-consuming and expensive activities, especially if the staff does not have adequate experience.^{1,2}

Reimbursement: The reimbursement for molecular tests varies greatly among tests and among states. Most commonly used molecular tests do now have CPT codes established, but reimbursement rates may vary as third-party payers generally reimburse molecular-diagnostic tests at a higher rate than other traditional test methodologies.

Finding answers

Moving toward in-house molecular testing is a complex decision, and it is made even more so by the scarcity of independent and reliable evaluations of assays and instruments. The published data available is likely to become quickly outdated due to the field’s rapid evolution and the frequent introduction

of new methods and suppliers.

Some resources for laboratory directors, however, are readily available. Ask colleagues at other labs about their experiences, attend meetings and conferences, and search the Internet for up-to-date information.

The challenge — which is both exciting and grueling — is that the field continues to grow rapidly. Technological advances will offer new opportunities to serve our patients and clinicians and a constant flow of new options about how to do so.

In the coming years, we can expect, for example, that multiplex molecular tests for detection of viral infections will probably replace viral culture and become the gold standard. We can also expect that new-instrument vendors and assay suppliers will appear, while some current providers could falter. Keeping abreast of who offers what — and what is next in each supplier’s product pipeline — adds yet another layer of complexity to the decision making.

In short, the difficult choices that the laboratory director faces today will need to be faced again, and with some regularity, but each step forward means better care and more cost-effective tests for patients. □

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