“Stat” Multiplex Polymerase Chain Reaction

Its Role in Clinical Microbiology

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In the laboratory, the department most affected by the introduction of molecular diagnostic tools has been the clinical microbiology laboratory. For organisms such as *Clostridium difficile*, polymerase chain reaction (PCR) is the gold standard for diagnosis. Incorporation of molecular diagnostic techniques into microbiology laboratories of differing sizes has been hastened by the development of simplified technological methods, individual cassettes incorporating all reagents necessary for diagnosis, and instruments allowing multiple tests to be run at the same time. Companies, such as Cepheid (Sunnyvale, CA), with its GeneXpert technology, even advertise that their tests can be run in a physician’s office. Pressure by the US Food and Drug Administration (FDA) on companies such as Roche has pushed them to change their offerings in molecular diagnosis from analyte-specific reagent kits to a commercially prepared, prepackaged assay with detailed instructions for performance of the test. This change has hastened adoption of molecular methods into the testing menu of laboratories that do not have the resources and staff to perform the extensive and time-consuming validations of assays that are not approved by the FDA.

Molecular methods have probably found their greatest use in microbiology laboratories in the diagnosis of viral infections. The reasons for the movement to molecular methods in this arena are varied. The rapid assays for agents such as influenza A and B and respiratory syncytial virus have low sensitivity and specificity. Direct fluorescent antibody assays require trained technologists and are generally only available during the daytime and early evening hours. Culture is less sensitive than molecular assays. The main advantage of molecular assays over conventional methods is that they can detect newer viruses that are often unculturable. Multiplex assays that detect multiple organisms in a single procedure are joining singleplex tests that detect nucleic acids from a single species. However, even the newer multiplex PCR tests, which have high sensitivity and specificity, are complicated, are technically demanding, and require highly specialized technologists. The turnaround time for these assays is fairly long (ie, 6-8 hours). As a result, these tests are generally only performed during daytime hours in virology/molecular laboratories in large, tertiary medical centers.

In this issue of the *Journal*, Xu and colleagues have demonstrated the advantages of using a multiplex PCR assay to diagnose respiratory viral infections in a core laboratory serving a patient population presenting to the emergency department. Performance of the panel required a hands-on time of 5 minutes and an instrumentation time of 65 minutes. The assay could be handled by general medical technologists with thorough training in both the procedure itself and the principles underlying the assay. As the investigators stated, published studies indicate that FilmArray, the system used in this study, had results similar to those of other molecular tests that detect viral respiratory agents. However, besides being faster, this system was more sensitive than some of the conventional molecular assays. FilmArray is a small desktop, closed single-piece flow, real-time PCR system that uses a nested PCR design. The instrument for this system can handle only 1 test at a time, so that, with the final testing volume, which peaked at 44 assays/d, the investigators were using 3 instruments with a fourth as backup. At the time of the study, the panel was designed to detect 15 respiratory viruses. The latest iteration of this FDA-approved assay detects an additional 2 viruses and 3 bacteria—*Bordetella pertussis*, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae*. 
Use of this assay produced shorter turnaround times and better patient care due to earlier treatment of influenza, a decrease in workup and therapy of nonviral agents, and reassurance of patients and clinicians. One fifth of the tests performed in this study were for the purpose of effectively cohorting patients for isolation. The FilmArray also has been used to detect respiratory viral infections in immune-compromised patients\(^3\) and children.\(^3\)

Pathologists considering use of the FilmArray in their laboratories may wish to ponder the disadvantages of this system. In some studies,\(^3,4\) the system was less sensitive in detecting adenovirus than the molecular assay to which it was being compared. Moreover, if consideration is given to using the system for detecting respiratory viruses in immunocompromised patients, herpes simplex and cytomegalovirus, both of which can cause respiratory diseases in this group of patients, are not included in the panel. Does the poorer detection of adenovirus and lack of herpes simplex and cytomegalovirus in FilmArray imply that a complete respiratory viral panel for immunocompromised patients includes the FilmArray plus other methods of detecting these 3 organisms? As mentioned by the authors in the study, other weaknesses include the inability to separate rhinovirus and enterovirus and the lack of quantitation for determination of viral loads. The possibility that a positive result may indicate prolonged shedding of a virus such as rhinovirus instead of acute infection is also a drawback of this and other multiplex systems. On the purely technical side, there is a low throughput and, for backup purposes, a minimum of 2 instruments is required. In addition, even in a Toyota Lean laboratory, such as the one in which this study was performed, the lack of an interface for computer data entry necessitated the use of 2 technologists for this function. This introduces the possibility of data entry error. Finally, the incorporation of a method for detecting \(B\) \textit{pertussis} into this assay raises the question of “pseudoepidemics” of this pathogen. Because of this possibility, the Centers for Disease Control and Prevention recommends that, during a suspected outbreak, positive PCR results should be confirmed by culture. The product literature for the FilmArray respiratory panel indicates that the target for detection of \(B\) \textit{pertussis} is the toxin gene, which is shared by other species of \textit{Pertussis},\(^5\) so false-positive results may occur.

The use of “stat” multiplex PCR assays in the clinical microbiology laboratory will surely increase. Fluorescence in situ hybridization using peptide nucleic acid probes performed on blood cultures to distinguish \textit{Staphylococcus aureus} from coagulase-negative staphylococci has reduced mortality, length of stay, hospital costs, and the use of vancomycin.\(^6\) However, optimal use of this technology means that individuals trained in fluorescence microscopy should be available 24 hours per day, 7 days per week. A FilmArray panel that can identify more than 25 pathogens and 4 patterns of resistance is presently going through the process for FDA approval. In a study using a developmental version of the panel,\(^7\) the assay identified 91% of 92 pathogens identified by the panel and detected all culture-proven methicillin-resistant \(S\) \textit{aureus} and vancomycin-resistant enterococci. The results of the study in this issue indicate that the development and use of different versions of these technologies will allow us to do a better job in performing our primary mission: providing optimal care for our patients.

References


