Molecular testing has been routinely used in clinical microbiology and infectious disease for almost three decades. In the past, a single probe, specific for the intended microorganism, was the diagnostic aim of the test. In present-day medicine with its complicated computer software programs, the molecular world has progressed to the point of sophisticated multiplex testing. This type of testing can include only a few pathogen targets or thousands with the use of microarrays. These multiplex tools allow the detection not only of the common pathogens but also emerging and newly discovered agents of infectious disease. This is especially true for zoonotic infections or those that cross from other species (e.g., severe acute respiratory syndrome [SARS]). In addition, finding co-infections with these new tools will allow therapy to be targeted more appropriately and improve the efficacy of patient care. There may often be a greater morbidity or mortality associated with such co-infections.

Some of the molecular platforms being developed for multiplex testing are real-time polymerase chain reaction (RT-PCR), gene and protein chips (microarrays), flow cytometry, and mass spectrometry. Factors that should be considered in selecting a molecular platform are performance characteristics, instrumentation and reagent cost, throughput, and specimen type.

Acute respiratory disease is the most prevalent disease in the world. Morbidity includes the common cold, influenza, bronchitis, croup, and pneumonia. The disease severity depends upon the patient risk and the type of virus, and there may also be an associated high mortality (e.g., transplant patients). Therefore, early and specific detection is paramount to both prevention and control. Early, specific, and comprehensive detection is part of the rationale for submitting multiplex, RT-PCR tests to the Food and Drug Administration (FDA).

FDA-Cleared Tests

Currently, there are two FDA-cleared test kits for respiratory viruses, and others are in various phases of the FDA clearance submission and review process. The first FDA-cleared RT-PCR assay, ProFlu+™, was developed by Prodesse (Madison, Wisconsin). The ProFlu+™ Assay is a multiplex RT-PCR in vitro diagnostic test for the rapid and qualitative detection of influenza types A and B viruses and respiratory syncytial virus (RSV). An internal control (IC) is included in the reaction mix to control for sample test inhibition. This closed-tube test generates a result from extraction to detection in about 3 hours. Another FDA-cleared test developed by Prodesse is specific for human metapneumovirus and has the advantage of using the same IC, so that a single nucleic acid extraction can be used for detection of all four viruses. A multiplex assay for parainfluenza virus types 1–3 is also in the last stage of clinical trials before submission to the FDA in the spring of 2009.
The other FDA-cleared test for the direct detection of respiratory virus in clinical specimens is the xTAG™ Respiratory Viral Panel (RVP; Luminex, Austin, Texas). The xTAG RVP detects 12 respiratory viruses: influenza type A, influenza A subtype H1, influenza A subtype H3, influenza type B, RSV subtype A, RSV subtype B, parainfluenza type 1, parainfluenza type 2, and parainfluenza type 3, human metapneumovirus, rhinovirus, and adenovirus. This open-tube system can generate a result in approximately 6.5 hours. Luminex technology uses up to 100+ color-coded beads (i.e., microspheres); each bead is coated with a specific reagent. The beads are then detected in a system that uses a combination of flow cytometry, lasers, digital signal processing, and traditional chemistry.
For Research Use Only

Several other multiplex, molecular respiratory tests are in development and at present are labeled For Research Use Only (RUO). Most detect a greater range of respiratory pathogens and offer a more comprehensive all-in-one approach. One such assay is the ResPlex™ II Panel using QIAplex™ and xMAP® technologies on the Qiagen LiquiChip System (Qiagen, Germantown, Maryland). An IC for inhibition and 18 targets can be co-amplified in the same reaction. The respiratory pathogens detected are RSV subtypes A and B, influenza types A and B, para influenza types 1–4, human metapneumovirus types A and B, Coxackieviruses/echovirus, rhinovirus, adenoviruses B and E, coronaviruses (NL63, HKU1, 229E, OC43), and bocavirus. Several newly discovered viruses, including human metapneumovirus, coronaviruses NL63 and HKU1, and bocavirus are already in the test menu.

Other companies are also developing tests for respiratory pathogens. EraGen Biosciences (Madison, Wisconsin) offers the MultiCode-PLx Respiratory Virus RUO Panel (RVP). This assay uses RT-PCR and the MultiCode-PLx Technology with Luminex detection and includes a 17-virus panel. Another company, Seegene (Rockville, Maryland), is developing Seeplex®, a respiratory pathogen 18-plex test. The test uses PCR and capillary electrophoresis to detect 13 viruses and 5 bacterial pathogens in a pneumonia panel. Idaho Technology, Inc. (Salt Lake City, Utah) offers an RUO respiratory RT assay capable of multiplex detection of 17 viruses and 4 bacterial pathogens. It has developed a FilmArray system by using a closed system (small pouch) and nested PCR to enhance sensitivity.

Pan-Viral and Pan-Bacterial Systems

Thus far, this discussion has involved the simultaneous detection (multiplex testing) of specific respiratory pathogens, including both bacteria and viruses. The real test for clinical molecular diagnostics is the development of high-performance pan-viral and pan-bacterial systems. These systems are currently in development and have shown great potential in detecting not only the common pathogens but also emerging and newly discovered agents of infectious disease. The Virochip (University of California, San Francisco) is an example of a pan-virus assay that was used for identifying the SARS virus, a newly discovered deadly coronavirus. This system not only achieves results in a short time but also uses a single spot on the chip to perform entire genome sequencing. Another pan-virus microarray, GreenChip, employs the same principle to achieve the same high-throughput sequencing. The final result is arrived at by a combination of complex algorithms and statistics to identify the pathogen.

Improving Patient Care

The ultimate question is, How can these eloquent, complex, and costly systems be used to improve the quality and lower the cost of patient care? The answer lies in the early and specific detection of the pathogen and subsequent cost savings on the part of the patient and health care provider. The recent acquisition (December 2008) of Ibis Biosciences by Abbott Laboratories may offer such a test in the near future. The Ibis technology involves the use of a universal biosensor (Ibis T5000™ Biosensor System). It is currently listed as an RUO, but has the potential of identifying 1,400 potential pathogens in a specimen without culturing or knowledge of the specific pathogen(s). The pathogen can be either bacterial or viral in origin, and bacterial or viral load-type quantitation is also possible. The system consists of a multiple PCR format linked to electrospray ionization time-of-flight mass spectrometry. Time to detection is about 5 hours, and there is the advantage of no additional cost for disposables.

Pathogen detection is only part of the laboratory testing for infection by a microorganism. Determining which antimicrobials to use is the reason culturing is still necessary in many cases. However, genes and mutations that confer antimicrobial resistance are being discovered and incorporated into many molecular tests. The presence of specific virulence factors (i.e., associated genes) is also important in the optimal management of the patient. *Staphylococcus aureus* (e.g., MecA, PVL, TSST-1), *Clostridium difficile* (e.g., Tox A and B), and vancomycin-resistant *Enterococcus spp.* (e.g., Van A) are examples of pathogens for which resistance and virulence gene detection is critical to patient care.

The newer multiplex molecular methods are not intended to replace classic phenotyping methods at present, but are expected to complement them. There are many reasons to use multiplex, molecular testing in the clinical microbiology laboratory: molecular test performance surpasses classical methods; pathogen detection is rapid; and hospital length-of-stay is reduced (i.e., cost savings). These assays have truly revolutionized the type of testing used in the clinical microbiology aboratory and have dramatically improved the quality of patient care.

Eragen Biosciences: www.eragen.com
IBIS Biosciences: www.ibisbiosciences.com
Idaho Technologies: www.idahotech.com
Luminex Molecular Diagnostics Inc.: www.luminexcorp.com
Prodesse: www.prodesse.com/USA/product/usIVD.html
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