

CSI Microbiology: Emerging Pathogens and a Staged Strategy for Detection and Discovery

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(See the article by Renwick et al., on pages 1754–60.)

The changing landscape of infectious diseases over the past 2 decades has astounded the medical community and the public. Emerging pathogens that cause new diseases (e.g., severe acute respiratory syndrome–associated coronavirus [SARS-CoV]), newly recognized microbial agents of known diseases (e.g., human metapneumovirus), and rapidly evolving pathogens (e.g., influenza viruses) all contribute to this seismic shift. A rate-limiting factor in our efforts to keep up with diseases caused by infectious pathogens is the absence of rapid, efficient, and affordable methods for microbial identification. Improving our strategies for diagnosing infectious etiologies will hold the key to our future relationship with infectious agents.

Acute respiratory infections are, globally, the major cause of mortality in children <5 years of age. The advent of diagnostic platforms suited to surveillance as well to clinical application will reduce the morbidity and mortality of respiratory

disease, both by enabling target selection for vaccine and drug development and by guiding patient care. In recent years, there has been substantial progress in applying molecular biologic advances to respiratory virus diagnosis [1]; nonetheless, a major gap has been the lack of a cost-effective, systematic way to identify the causes of respiratory diseases in populations. The article by Renwick et al. [2] in this issue of the *Journal* makes 2 unique contributions to this field: it demonstrates the need to consider and identify rhinoviruses as a cause of serious acute respiratory disease in children, and it establishes the MassTag polymerase-chain-reaction (PCR) multiplex platform as a practical tool for microbial surveillance.

The MassTag PCR method, developed by Renwick et al. [2] and used in their study investigating the etiology of respiratory disease in hospitalized children, provides a paradigm for new detection strategies for early recognition and containment of a wide range of respiratory pathogens. This multiplex technology, in its first evaluation in 2005, was shown to be effective in identifying all the main respiratory viral pathogens, including respiratory syncytial virus, human parainfluenza viruses 1–3, metapneumovirus, influenza, and SARS-CoV as well as *S. pneumoniae* and *H. influenzae* [3]. In 2006, Lamson et al. [4], in the same group, went on to report the use of the method to investigate both undiagnosed

influenza-like illness in New York State and the discovery of a novel genetic clade within the picornaviruses; “human rhinovirus New York” was the first new agent to be detected by use of MassTag PCR.

Rhinoviruses have long been a relatively underappreciated cause of acute respiratory infection, a view that is beginning to change [5]. In their study in this issue of the *Journal*, performed in collaboration with the Robert Koch Institute (Berlin, Germany), Renwick et al. [2] report clear evidence that links these viruses to severe respiratory disease: 75% of viruses detected among 97 nasopharyngeal aspirates from children hospitalized with acute respiratory infection—with no pathogen identified by routine methods—were rhinoviruses. Independently, in publications following that by Lamson et al. [4], other investigators found similar evidence that associates severe respiratory disease with rhinovirus infection, implicating known serotypes of rhinovirus groups A and B as well as the same, novel viruses found in New York in pediatric lower-respiratory-tract infection [6] or asthma exacerbations [7]. With work now reported by 3 research groups employing different methods, the likely contribution of rhinoviruses to acute respiratory disease cannot be ignored and deserves further study.

The diagnostic strength of highly multiplexed systems, such as the MassTag

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PCR platform applied by Renwick et al., in the Lipkin group, builds on a series of advances by this group [2]. In 1987, the first application of subtractive cloning in microbiology led to the identification of a novel pathogen, Borna disease virus, the prototype of a new family in the order Mononegavirales [8, 9]. Another new strategy—namely, domain-specific differential display PCR—ultimately led to the recognition of West Nile virus as the cause of the outbreak of encephalitis in New York in 1999 [10, 11]. The recent development of pathogen microarrays, combined with a comprehensive panmicrobial sequence database, has added to the repertoire of methods for sensitive and broad differential diagnosis of infectious disease [12, 13] and has facilitated differential diagnosis of filovirus versus malaria in samples obtained during an outbreak of hemorrhagic fever. In early 2007, the same research group [14] pioneered the use of unbiased, high-throughput sequencing to assemble a comprehensive inventory of microflora from honeybee colonies affected by colony-collapse disorder [14].

The transition of cutting-edge pathogen-discovery technologies from research to clinical laboratories will not occur immediately. Nonetheless, it is not premature to plan for this in the near future. Key to implementation will be the development of a *staged strategy for pathogen detection* that enables low-cost and efficient differential diagnosis of infectious diseases [15]. One approach might be to begin with a multiplex PCR screen, such as the MassTag PCR-based assays described here. If the initial screen fails, microarrays could be em-

ployed. More labor- and resource-intensive unbiased, high-throughput sequencing would be reserved for the most difficult challenges.

Considerable emphasis has been placed on ensuring global access to vaccines and drugs for treatment of infectious diseases. Less attention has been focused on the importance of understanding microbiological data in the context of public health. To this end, efforts such as those undertaken by the Lipkin group, directed at training and equipping an international cast of investigators in state-of-the-art methods for molecular diagnostics, are critical. It is no surprise that the list of investigators collaborating on papers from their laboratory often reads like a United Nations of science. Such worldwide capability in global infectious-disease surveillance is important to local as well as international security. Commitment to technology transfer and global collaboration is essential if we are to have the agility required to keep pace with emerging infectious diseases. Pathogen surveillance and discovery can promote global interaction via collaborations on matters that know no national or political boundaries but simply reflect our common humanity.

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