The specialty of pathology must emphasize the unity of pathology without creating artificial boundaries. Enabling technologies are removing walls within our own specialty. In large and complex medical centers, we are at the center of immense change in medical practice. Pathologists must grasp many complex issues related to diagnosis, prognosis, and therapeutic monitoring. Pathology, as a field of medicine, is challenged in the breadth and the scope of medical practice, the growing volumes of patients, and the increasing complexity in patient care.

Figure 1 illustrates that we may consider microorganisms, genes within microbes, or human genes that may predispose one to infections. Ultimately we must integrate large amounts of molecular data if we want to lead change in medicine. Pathologists must be comfortable in the center of the diagnostic arena, while taking advantage of the tools and not letting the tools define pathologists.

Depending on various estimates, approximately 75% of medical decision making depends currently on imaging, laboratory tests, histology, and other information generated in clinical laboratories. About 5% of health care costs are attributed to laboratory tests, and we will likely see shifts in health care dollar allocation from therapeutics to diagnostics. Thus, pathology and laboratory medicine would be expected to play an even larger role in the future of personalized medicine.

E pluribus unum is Latin for “out of many, one.” We are now faced with many more questions in an increasingly complex world of medicine. We are basically trying to move from “slow roads” to “freeways.” The freeways must be expanded to effectively reach one diagnosis by highly parallel, multianalyte testing. How much information can we generate by parallel testing and how can we integrate the data? Ultimately from the many questions, probes, and possible microbes, can one obtain an accurate diagnosis with molecular methodologies? The answer may be one microbe, one gene, one mutation, but it may also be one set of mutations, one set of genes, one molecular pattern, or one genetic profile of integrated information that ultimately yields a meaningful diagnostic report.

The specific context of this presentation is medical microbiology, but the challenges apply to other areas including genetics, oncology, and pharmacogenomics. Medical microbiology and infectious diseases are replete with challenges. With all of the advances in antimicrobial therapies and diagnostic microbiology, we are faced with a variety of etiologies to consider: bacteria, parasites, fungi, viruses, new infectious agents, re-emerging pathogens, and underappreciated agents of infection. Fundamental questions include whether microbes are colonizers or pathogens and whether human beings have a core human microbiome or definable microbial community that is present in healthy individuals. Changes or variation in the human microbiome may affect a pathogen’s ability to cause disease, and disease susceptibility may depend on host genetic or developmental factors. The presence of Clostridium difficile in an infant simply represents colonization due to the relative paucity of toxin receptors, in contrast to older children and adults.

The limitations of current strategies in diagnostic microbiology include an overreliance on colonial morphology of cultured isolates and microscopic morphology (eg, Gram stain). The power of culture-based approaches is that we could pursue many possible infectious agents with rich media formulations that enable successful cultivation of many different microbial pathogens. The key point is that the advent of array-based testing in molecular diagnostics is enabling pathologists to pursue open-ended clinical questions with many probes. E pluribus unum can be applied in the modern era of molecular pathology and microbiology. Current examples such as molecular pertussis testing depend on tightly focused clinical questions. For situations with an established diagnosis such as human immunodeficiency virus 1 viral load testing, such a focused testing approach may be quite useful. However, if new molecular strategies will truly compete with conventional microbiology and culture, array-based testing must deliver on its promise for applications of molecular diagnostics to less focused questions and more general clinical challenges (eg, respiratory tract infections).

The “second” human genome project, otherwise known as the human microbiome project, has been recently launched. In May 2007, Elias Zerhouni, MD, director of the US National Institutes of Health, authorized the human microbiome project as a cross-institute roadmap project that may revolutionize biomedical research. The hu-
Figure 1. Convergence of diagnostic applications with emerging highly parallel molecular technologies. This schematic diagram illustrates how different areas of medicine and diagnostic pathology are depending on massive molecular datasets generated by more global and highly parallel testing platforms.

The human microbiome project includes analyses of microbial communities colonizing human skin and mucosal surfaces in the gastrointestinal tract, the oral cavity, the upper respiratory tract, and the human genitourinary tract. A more comprehensive understanding of microbial communities and fluxes in these communities may contribute to improved evaluation of human health status and differential susceptibilities to human disease. In a recent study from David Relman's group using metagenomics and high-throughput DNA sequencing, more than 60% of bacteria were completely novel and more than 80% of these sequences represented nonculturable organisms.

Is there a core human microbiome? Are there variabilities in that human microbiome that may have profound implications on disease susceptibility? The susceptibility to inflammatory bowel disease and other immune-mediated disorders are now being considered in the context of the human microbiome project. Evidence suggests that fluxes in microbial communities can have a major impact on the host immune response. The diagnosis of acute gastroenteritis provides a useful example of the challenge that we face today in clinical pathology. In a prestigious medical center in Seattle, only 47% of stool samples that underwent complete testing for viruses, bacteria, and parasitic agents yielded a specific etiologic agent and diagnosis. Approximately 50% of the time, the laboratory facilitates the diagnosis of infectious diseases with traditional methods. This study included nearly 5000 patients who were discharged with a diagnosis of diarrhea or acute gastroenteritis. Less than half of these cases yielded an answer using approaches such as viral antigen testing, viral culture, and bacterial culture. Whether we are challenged with respiratory tract infections, skin infections, or gastrointestinal infections, we face similar challenges in diagnostic microbiology. With a variety of strategies including, but not limited to culture, multiple single or oligo-analyte tests may not yield a diagnosis and may delay proper treatment.

As in gastrointestinal infections, a continually expanding repertoire of potentially infectious agents including a plethora of respiratory viruses have been discovered during the past 5 years. Recently discovered viruses include the severe acute respiratory syndrome coronavirus, human bocavirus, and human metapneumovirus. We are just beginning to appreciate the complex interplay of viruses, other microbes, and the host immune response. How do we address the fundamental issues of too many genes and microbes in the human genome and microbiome, respectively? Molecular tools can be used as gene- or analyte-specific tools to get specific answers. However, too many...
analyte-specific tests yield a massive, cumbersome, and expensive diagnostic enterprise. The transition from targeted technologies such as analyte-specific polymerase chain reaction (PCR) and real-time PCR to more global strategies such as PCR arrays, liquid bead arrays, microarrays, and high-throughput DNA sequencing is beginning to occur in laboratory medicine. Parallel testing technologies are available today for pathologists to design new types of disease-oriented assays. A large-scale shift toward massively parallel and ultra high-throughput technologies is occurring already in biomedical research. The implementation of parallel testing and high-throughput technologies for the first time in the diagnostic laboratory is changing the future landscape or “futurescape” of clinical pathology.

The application of relatively global molecular diagnostic strategies in areas such as oncology, pharmacogenomics, or infectious diseases is a central theme in the current transitional era of molecular pathology. Arrays are a key example of new strategies for parallel, multianalyte testing. This term (arrays) applies to any surface or solution containing many antibodies or probes that recognize specific proteins (antigens) or nucleic acid sequences in parallel. Current procedural terminology billing codes have intentionally used the more general term, arrays, because this term is more inclusive of a variety of strategies that may be useful for diagnostic testing. As pathology enterprises consider more highly parallel strategies, the distinction between arrays and microarrays becomes potentially important. The term microarrays refers to high-density solid phase based arrays that usually require optic and computer-assisted signal detection. Array formats have also been extended to liquid phase in the form of liquid bead arrays.

Multiple opportunities for potentially disruptive technologies facilitate multianalyte detection and parallel testing in laboratory medicine. As one example, liquid bead array technology (eg, Luminex Corp, Austin, Texas) is being applied in different ways in infectious diseases including the diagnosis of respiratory tract infections and potentially other infections. In this technology, the beads labeled as microspheres are essentially labeled with either antibodies or oligonucleotides to serve as probes for proteins or nucleic acids. Signal detection of a target uses microspheres with different internal color codes that establish the identity of each probe and, hence, analyte. A modified flow cytometer essentially detects the analyte and provides quantitative information based on signals emanating from secondary nucleic acid probes or antibodies. One may combine the ability to detect specific cells or proteins with DNA or RNA targets by combinations of multicolor flow cytometry with molecular diagnostics.

Liquid bead array technology formed the basis of the xTAG Respiratory Viral Panel (Luminex), the first US Food and Drug Administration–approved molecular array-based test for infectious diseases. This US Food and Drug Administration–approved test includes 12 different viruses, and this approach could be extended to include more viruses and infectious agents in the future. The US Food and Drug Administration has already approved microarray-based pharmacogenomic testing based on human DNA polymorphism detection (Affymetrix Inc, Santa Clara, California). Groundbreaking developments in the applications of liquid bead arrays and microarrays to the challenges of infectious diseases signal the shift to global, highly parallel molecular testing in microbiology. A variety of potential strategies have been introduced into liquid bead array formats, resulting in different methods for linking DNA probes or antibodies to beads or microspheres. Signal generation is based on fluorescence detection with multiple potential tags or dyes. Target amplification methods such as PCR can be combined with highly parallel signal detection approaches such as liquid bead arrays and microarrays.

Two recent publications addressed the issue of multianalyte testing in infectious diseases. The study by Lee et al3 applied liquid bead arrays to nasal washes from 5-year-old children. This group demonstrated the application of microsphere flow cytometry to generate specific signals for any one of more than 20 different viruses. The Respiratory Multi-Code-PLx-Assay as applied by Lee et al3 detected respiratory viruses in approximately 72% of clinical specimens versus approximately 23% of the specimens by the conventional methods of virus culture and direct fluorescence assay. Molecular array-based testing yielded tremendous improvements in the ability to get an answer in a timely and accurate manner. The study by Mahoney et al4 yielded similar results. Liquid bead arrays, using target-specific primer extension, demonstrated a major improvement in sensitivity with this highly parallel technology (98.5% vs <70% sensitivity) in mostly adult patients.

A variety of array-based or sequencing-based approaches are being applied to molecular bacteriology. In blood cultures, typically 10 to 20 hours may be required to get a positive signal in cases of bacteremia or fungemia. Microorganisms can be visualized by Gram stain following culture, but morphology does not yield a definitive identification. An additional waiting period of 1 to 2 days is required for subculture, biochemical identification, and antimicrobial susceptibility testing. Molecular technologies may be applied to identify pathogenic microbes directly in positive cultures using sequencing or array-based approaches. One could proceed directly from primary culture and Gram stain to automated DNA extraction. Following automated nucleic acid extraction, various molecular strategies including real-time PCR arrays, liquid bead arrays, and DNA sequencing may be deployed to establish more accurate and timely diagnoses. High-density microarray technology can be applied to detect a variety of infectious agents (viral, bacterial, or parasitic agents) or human genes and polymorphisms that may confer susceptibility to infectious diseases.

Microarrays are being used to identify new viruses as well as to develop new diagnostic algorithms. Mathematics can be applied by deploying algorithms for comparing hybridization patterns with predicted energy profiles (Figure 2, A and B).5 Using computational methods, signals can be compared for ranking viral identities in the laboratory—e pluribus unum in action. The Virochip highlights the application of microarray technology to virus discovery,6 and clinical applications of diagnostic viral microarrays are emerging from these seminal efforts.

This Virochip strategy enables detection of more than 140 different viruses with high-density DNA microarrays. In this case, Wang et al8 detected human rhinoviruses and paramyxoviruses in nasal lavage specimens. In this case the human rhinovirus signal is observed in a lavage from a patient with experimental rhinovirus infection (visible at day 2 of infection). Human rhinoviruses were also detected in individuals with natural colds; virus-specific
patterns were detected with different probes for each rhinovirus and parainfluenza virus. Specific molecular microarray patterns may finally replace the virologist’s reliance on immunofluorescence, antigen testing, and culture.

The Phylochip was developed at Lawrence Livermore Laboratory in Livermore, California, and is now being used in the context of human microbiome research. More than 8000 organisms per chip are represented on the array. Such microarray strategies facilitate pathogen discovery and detection of potential microbial fluctuations associated with health and disease. By using comprehensive high-density microarrays, new molecular patterns of diagnostically relevant information are becoming apparent.

Flanagan et al applied the Phylochip to a group of intubated patients colonized with Pseudomonas aeruginosa and hospitalized in the intensive care unit setting. Patients were monitored on antibiotic therapy with a comprehensive microarray-based, Phylochip strategy. The reduction in microbial diversity was correlated with the pattern of disease and outcome. Antimicrobial therapy yielded a reduction in microbial species diversity from an average of 16.2 bacterial species without antibiotics compared with an average of 5.6 species with antibiotic therapy. The microbial diversity index was markedly reduced, possibly contributing to more severe infection and disease. In a variety of recent studies, loss of microbial diversity has been
correlated with predilection to human disease. Again, the technologies enable the acquisition of new patterns and profiles of molecular data visualized in new ways.

New combinations of bacteria and fluxes in microbial populations at nonsterile sites can be examined for the first time using global molecular approaches. Ultimately one profile or a highly complex pattern may deliver meaningful information.

In tracking the presence of *Pseudomonas* pathogens, even if *Pseudomonas* organisms were present and did not vary, the loss of bacterial diversity was correlated with development of ventilator-associated pneumonia.7 Microbiologists and clinical pathologists are beginning to think about fluxes in aggregate microbial populations and not just the presence or absence of single pathogens. Antimicrobial therapy and combinations of antibiotics may be refined in the future on the basis of a comprehensive assessment of the human microbiome.

Weston and Hood8 nicely articulated the concept of systems biology and how it applies to medicine, with the idea of multiparameter diagnostics. Disease susceptibility could be predicted more faithfully based on an enhanced knowledge of the human microbiome and human genome. The microbiome is a fundamental part of humanity, comprising 90% of our total cells and including 10^{13} bacterial cells (vs 10^{11} human cells). Information sets can be combined to predict disease susceptibilities in new ways. Diseases may be stratified in terms of human genotypes and combinatorial fluxes in human gene expression and microbial populations. Highly parallel technologies may be able to more effectively monitor treatment responses, to examine quantitative shifts of viruses and other pathogens, and to make more sophisticated predictions of therapeutic responses.

Where are we headed? Multiparameter and highly parallel diagnostic testing will hasten the removal of tool-based boundaries within pathology, while facilitating the integration and consolidation of massive datasets. The paradigms continue to change in molecular pathology.

In gene expression and proteomics profiling, we need to consider less and minimally invasive approaches. Body fluids and blood serve as windows of opportunity to get a diagnosis more easily and effectively. Maybe in vivo imaging will be combined with sampling of certain body fluids or certain mucosal sites that are readily accessible. Keep in mind that the relative costs of sequencing (per kilobase) and the costs of doing arrays are decreasing, so costs per gene/microbe/analyte will dissipate during the next decade. However, the counterforce is that higher expectations will be in place regarding volumes of DNA sequencing and array-based testing for microbial genomics and metagenomics.

We need to think differently about teams of pathologists and physicians in the diagnostic enterprise. We also need to consider how teams are designed within departments of pathology as partnerships of technical and professional staff. Effective teams of pathologists, medical technologists, and informatics specialists need to come together to address the challenges of highly parallel testing in molecular pathology.

This issue of medical technologists and labor shortages was raised by Jennifer Hunt, MD. In addition to qualified personnel, molecular automation strategies must be confronted so that medical technologists and pathologists effectively generate much more data and redesign their respective roles and work teams. New technologies will help to drive processes so that pathologists can be designers as well as operators.

In conclusion, analyte-specific molecular methods will continue to be useful in clinical situations with targeted questions and a limited differential diagnosis. The shift to multianalyte testing and global molecular strategies will continue to occur at a rapid pace in pathology. Our specialty must consider how to integrate the immense amount of information for patient diagnosis and treatment monitoring. Which key 100 genes or 100 microbes are important biomarkers within a group of patients that deliver clinically meaningful information? Finally, a variety of analytes may be evaluated in parallel to deliver important answers regarding diagnosis and prognosis or assessment of treatment responses.

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References