L. Barth Reller and Melvin P. Weinstein, Section Editors

Implications of New Technology for Infectious Diseases Practice

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New assays for the diagnosis of infectious diseases—particularly those that use molecular technologies—will revolutionize infectious diseases practices, but the fulfillment of the promise is several years away. Problems with currently available molecular assays include a lack of knowledge about the extent of microbial nucleic acid in "normal" hosts, concentration of agent material in small volume samples, lack of microbiologist expertise, lack of adequate reimbursement, and difficulty with validation based on conventional methods. Clinicians must appreciate the shortcomings of new technology to use it effectively and appropriately. However, we are realizing tangible progress in our ability to detect new etiological agents; the availability of rapid, accurate diagnostic tests for previously difficult infections; and advances into new, human response–based paradigms for diagnostic testing.

The promise of a Star Trek–inspired hand-held infectious diseases diagnostic device that scans a patient's body and immediately provides the information needed for diagnosis and treatment is probably years away. Until that time, when clinicians and microbiologists become unemployed, we can use the amazing technologies that are already available. This overview will discuss a selection of technologies and provide an opinion about their utility, their pitfalls (summarized in table 1), and their potential. Although the day when all diagnostic microbiology laboratories will routinely use molecular tools has not yet come [1, 2], many clinicians believe that a plethora of such tests is readily available. Laboratorians are regularly asked to perform a PCR test to detect an agent that only a few published research studies have reported, with those studies usually describing only preliminary results. Physicians often seem surprised that the laboratory cannot provide this service.

A survey by the American Society for Microbiology published in 2003 provides a snapshot of the current environment in the United States. Of the 612 laboratories (representing community, academic, and commercial laboratories) that responded to the survey, 95% performed bacteriology tests, but only 17% performed any molecular tests for infectious diseases [3]. The bulk of the latter laboratories offered only molecular testing for *Chlamydia/Neisseria gonorrhoeae* (as determined on the basis of College of American Pathologists Surveys participant summaries). For the foreseeable future, because of staffing and financial issues, many nonconsolidated or nonacademic hospital laboratories will not perform new, high-technology, expensive, and skill-demanding assays.

There is a national shortage of clinical laboratory scientists. The number of training programs has decreased over the past decade, and qualified students are now choosing careers in medicine or computer science. Nurses are paid dramatically more than clinical laboratory scientists—a further disincentive for students to consider a laboratory career—although the same level of professionalism and dedication to patient care is required. The overall vacancy rate among microbiology technologists is 57%, and almost one-half of all vacancies require at least 3 months to fill [3].

The staffing shortage in the pipeline in the United States today becomes more worrisome when combined with the aging of the current experienced staff. Thirty-four percent of microbiologists working today are >50 years old [3]; most of these will retire in the next 10 years. Their competence in molecular methods is miniscule. However, these senior microbiologists can interpret a Gram stain and deliver an educated guess as to the identification of a pathogen at an early stage. Infectious diseases clinicians have relied on these expert workers, and

Received 25 March 2006; accepted 31 July 2006; electronically published 10 October 2006.

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Clinical Infectious Diseases 2006; 43:1318–23

1058-4838/2006/4310-0016$15.00

Table 1. Key problems relevant to new technology.

Reliable molecular diagnostic tests are not readily available for many infectious agents
Commercial tests should only be used for validated specimen types
Transportation problems, low concentrations of infectious agent, primer binding site genetic changes, final assay volume, inhibition, contamination, nonspecific amplification, and operator error lead to false-negative and false-positive amplification results
Genomic bacterial sequencing is subject to error because of sequence homology among different bacteria, database problems, and mutations
Unknown extent of microbial DNA in "normal" host tissues
Lack of necessary resources (human and economic)
Reimbursement often not sufficient to cover costs

today's infectious diseases trainees are still being trained in laboratories utilizing such resources. The new, younger laboratory workforce is not highly skilled in either the conventional or the high-tech environment, and microbiologists in general are in short supply. We are facing a knowledge and practice gap for the next 10–15 years, during which time molecular tools will gradually fulfill their promise. Even in those laboratories that are staffed with skilled microbiologists during the daytime, many critical test results are needed on the evening and night shifts, when the laboratories are staffed by nonspecialized laboratorians. As a result, physicians will have less trust in test results and will need to make a greater number of empirical decisions and use a greater number of broad-spectrum antimicrobial agents. Another aspect of the dearth of experienced technologists is that testing has become more instrument based than discipline based. This is apparent in the migration of hepatitis and HIV serological testing from microbiology or virology departments to the immunochemistry laboratory, where generalists or chemistry technologists perform these tests along with tests for other serum factors, such as toxins and proteins. The testers are, in general, not knowledgeable about the agents for which they are testing, which could lead to interpretation errors or failure to recognize inappropriate results.

It is difficult, at best, to validate test results (assuring the clinical utility, reliability, and reproducibility of results) using new technologies. Traditional culture- or antigen-based comparator methods often yield results that fail to correlate with the newer, more sensitive tests. Finding clinical criteria or alternate molecular targets to verify which result is correct can be tedious, expensive, and occasionally impossible. Obtaining adequate standards or positive patient samples to validate test results and to train performing technologists is also challenging. One example is PCR of CSF specimens for herpes simplex virus meningoencephalitis; this is considered to be the best diagnostic assay available, because cultures are insensitive, and brain biopsy is too invasive [4]. The difficulties in finding enough samples and in creating seeded samples with known viral loads for validation studies have prevented or delayed development of this and other tests in many laboratories [5, 6]. In fact, identical problems have impeded submission and US Food and Drug Administration (FDA) approval of commercial assays for herpes simplex virus. Some analyte-specific reagent test kits are available for both herpes simplex virus and enteroviruses, but they still require extensive in-house validation studies. The threat of an avian influenza pandemic may help spur the development of multiplex molecular assays for respiratory viruses [7]. The use of laboratory tests for diagnosis of enteroviral CNS disease was shown to be cost effective long ago [8], but the availability of rapid molecular tests for this purpose has been long coming. In many circumstances, tests that infectious diseases physicians have heard about (e.g., at national meetings, such as that of the Infectious Diseases Society of America) will be available only as send-out tests to referral laboratories, accompanied by the attendant transportation problems and possible specimen degradation, delayed results, and costs. If an available molecular test is not performed in the local laboratory, one of the most important benefits—rapid results—is not realized, because the sample must be sent to a distant reference laboratory. Even after the newest generation of real-time molecular test systems, such as the GeneXpert test for group B streptococci (Cepheid), slated for FDA clearance as moderately complex, becomes available [9], their initial cost may delay widespread implementation.

There are, of course, some widely performed molecular methods, such as HIV load [10] and nucleic acid amplification (NAA) assays for *Chlamydia trachomatis* and *N. gonorrhoeae* that use genital samples or urine specimens [11, 12]. However, many physicians assume that one can just as easily test an eye swab as a cervical swab, without appreciating that the laboratory must perform an extensive validation of a sample not included in the original FDA clearance of the device if a report is to be issued (and the test is to be paid for) [13, 14]. Many molecular tests are limited to reference laboratories or for research investigations only. Of course, not all new technology is nucleic acid based, and I will also discuss other methods.

PROBLEMS SPECIFIC TO AVAILABLE MOLECULAR DETECTION TESTS

A number of nucleic acid hybridization and amplification methods are now in use, including direct probe hybridization (AdvanDx FISH for *Staphylococcus aureus* [AdvanDx] and GenProbe for group A streptococci [GenProbe]), hybrid capture (Digene for human papillomavirus; Digene), PCR, branched-chain DNA (bDNA; Bayer Diagnostics), and transcription-mediated amplification (Probe-Tec for *Chlamydia* and *N. gonorrhoeae;* Becton Dickinson). Sample volume (which encompasses both original sample volume and number of nucleic acid sequences present in the final amplified sample portion), transport conditions, dilution factors, inhibitory factors, genome changes, instrument problems, and operator error all contribute to false-positive and false-negative test results [6, 15–18]. Even closed systems at the detection end do not prevent cross-contamination from positive sample processing at the front end. For example, current FDA restrictions require that Papanicolaou smears be first performed when a single suspension of cervical cells is received in preservative, with requests for both cytology and for detection of human papillomavirus, *Chlamydia* species, and *N. gonorrhoeae;* this contributes to the common finding that insufficient sample size remains for the infectious diseases tests after the Papanicolaou sample has been removed [19]. Unfortunately, unlike direct visual examinations in which sample quality can be assessed, a negative result of such an assay includes no comment on the quality of the specimen. Although molecular tests that use whole blood samples for detection of the agents of sepsis are not yet available, several such assays are in development. Volume will be a factor in the sensitivity of these tests. The amount of bacteria in blood obtained from a patient with bacteremia is often <1 cfu/mL [20], yet the volume used in most NAA reaction vials is <10 μL. Even if the sample is concentrated in some way, the chance that microbial genetic material will reach the reaction vial could be very small, resulting in false-negative results. In samples containing human neutrophils or erythrocytes—the very samples most likely to harbor the infectious agent—it is challenging to concentrate microbial DNA without also adding overwhelming amounts of human somatic DNA, and the available front-end methods (extraction and filtration columns, magnetic particle capture, and silica-based systems) still lack efficiency [21, 22]. Manufacturers are spending most of their energy on back-end technology (detection and reporting). Front-end processing is urgently needed, ideally resulting in the development of a creative new method for concentrating cell-rich specimens (such as purulent fluid, blood, and sputum) from humans to allow for sensitive detection of microbial nucleic acids.

Diagnosis of tuberculous meningitis is another example of the problems inherent in amplification technology using PCR [23]. Patients may have confounding signs and symptoms, the number of organisms in the CSF sample may be very low, and conventional tests are insensitive and/or slow. Direct acid-fast staining of CSF specimens has a sensitivity of ~52% [24]. The volume of CSF needed for adequate culture is at least 5 mL (preferably 10 mL), because *Mycobacterium tuberculosis* is extremely buoyant in CSF as a result of its waxy cell wall; thus, concentration by centrifugation is inefficient [25]. Smears for acid-fast bacilli made from the sediment must be layered and examined exhaustively—sometimes for 30 min—in the hope of detecting the rare *M. tuberculosis* rod [23]. NAA would seem to be the answer. Sadly, NAA tests display an overall sensitivity of 71% (range, 25%–100%) and a specificity of 95% (range, 92%–97%), as determined by a meta-analysis of 49 published studies, 35 of which used home-brewed (individual laboratory–developed and –validated) methods [26]. When results from the 14 laboratories that used commercially available assays were evaluated separately, the overall sensitivity decreased to 56% (range, 46%–66%). These are the assays that are likely to be available to most clinicians. The NAA results were equal to those for the acid-fast bacilli smear. Clinicians must realize that unwanted reliance on new technology may lead to diagnostic errors.

Another highly favored technology for laboratory diagnosis of infectious diseases, whether testing tissue directly or testing isolated colonies, is identification by nucleic acid sequencing. There are also pitfalls in this approach. For example, many organisms, although they may be antigenically or biochemically distinct, are virtually genetically identical. Members of the *Bacteroides fragilis* group, for example, are highly related and cannot be separated easily by standard sequencing methods [27]. Most clinicians realize that *Escherichia coli* and *Shigella* species are highly related [28], but they have not considered the difficulty that this relatedness would pose if we relied on sequencing to identify these genera directly in patient samples. The extent of shared genes and taxonomically (versus phenotypically) shared traits among most microbes is unknown.

Another issue that vexes clinicians is the taxonomic confusion caused by genetic investigations. *Rhinosporidium seeberi,* for example, is an agent of mass lesions in the sinus and mucous membranes. This organism has often been observed in histopathologic sections, but it has never been grown in culture. It resembles the spherule of *Coccidioides immitis,* however, so it has long been considered an "unculturable" fungus. Recently, 18s rRNA sequencing placed the organism into a group of protozoan fish parasites [29]. We are constantly faced with new names for familiar organisms—and even with new organisms that may or may not be important in familiar syndromes. Before more-extensive testing was performed, the newly discovered *Mycoplasma fermentans* (originally called *Mycoplasma incognitus*) had been implicated in both Gulf War syndrome and HIV infection [30, 31]. It was later found to be quite common

in humans [32]. After effective therapy, results of PCR of genital samples obtained from patients with *C. trachomatis* remain positive for >2 weeks [33]. Nucleic acid is detectable but probably not viable. We do not know the extent of carriage of unculturable microbial or "normal" microbial DNA in other healthy hosts.

NONTECHNICAL PROBLEMS WITH MANY NEW TECHNOLOGIES

The cost of equipment, expensive reagents, and additional skilled personnel needed is not easy to justify. For example, the FDA has cleared a real-time PCR test for group B streptococcal vaginal/rectal colonization during labor [34]. Widespread use of this test could negate the need to perform cultures for pregnant women during weeks 35–37 of gestation, and it would solve the problems associated with treating women who present in labor without screening test results [35]. Currently, 25% of women receive prophylactic penicillin during labor [36]. This number would decrease if only women with PCR results positive for group B streptococci at labor were treated [36, 37]. The problem, however, is that current recommendations [38] have already reduced the incidence of early-onset neonatal group B streptococcal disease to less than the 2010 National Health Objective of 0.5 cases per 1000 live births, and additional improvement is not cost-effective [39]. Lucile Packard Childrens Hospital Johnson Center, a Stanford University Medical Center affiliate on the same campus, delivers ~1800 babies annually. To perform the PCR assay immediately after each sample is received (a necessity to assure that prophylaxis is administered for at least 4 h before delivery), we would have to staff 1 technologist at all times. Test performance takes ~15 min of hands-on time, and the test itself runs 45 min, so a technologist must perform continuous sample management. Twenty-four/seven staffing for 365 days per year would require 3.9 clinical laboratory scientists, at a labor and overhead cost of $316,875. The reagents—not counting the cost of the instrument itself—cost $46,800 annually, for a total yearly cost of $363,675. Stanford University Medical Center (Stanford, CA) patients have a colonization rate of 17% among women in labor, resulting in 306 babies at risk per year. To prevent 3 potential group B streptococcal infections using this test, the cost per infection avoided would be $121,225. This is a hard sell to hospital administrators who are focused on the bottom line. Potential laboratory staff labor savings with the use of random-access, moderately complex molecular systems, such as GeneXpert (mentioned above), may help to bring down costs.

Costs for new technologies are often added onto existing testing, so overall diagnostic costs increase. Centers for Medicare and Medicaid Services reimbursement is lagging dramatically behind the use of new tests. In the northern California reimbursement area, our laboratory receives only $98.08 for a PCR test for *Bordetella pertussis* and *Bordetella parapertussis,* although it costs the laboratory more than $150 to test each sample, because the tests are always performed one specimen at a time, which necessitates testing an additional 3 controls simultaneously.

CHALLENGES AND SUCCESSES ASSOCIATED WITH MOLECULAR AMPLIFICATION AND SEQUENCING

The future is not all bleak, of course. New agents of important diseases are being discovered with the help of molecular technologies. Among those to have been elucidated recently are *Tropheryma whipplei, Bartonella henselae, Ehrlichia chaffeensis,* and *Mycoplasma genitalium* [40]. Infectious disease–related challenges are ready for solutions with our new molecular tools. The etiology of 42% of cases of pharyngitis is unknown [41], and the etiology of 20%–40% of cases of diarrhea is unknown [42], as is the etiology of 40%–60% of cases of pneumonia [43]. We cannot cultivate ~25% of the organisms in the subgingival crevices of patients with periodontal disease [40]. All of these mysteries regarding vexing syndromes will eventually yield to molecular technologies.

MICROARRAY TECHNOLOGY

Proteomics (i.e., profiling the proteins generated by human cells in response to various stimuli, including components and products of infectious agents) is in its infancy, but exciting developments are enticing. The spots of DNA or RNA on "gene chips" can be designed to be homologous with nucleic acid sequences in the sample, representing nucleic acids found in both the normal situation and the activated state of transcription or representing various genetic sequences unique to a vast array of microbes for detection of infectious agents [44]. Samples are treated to break down their DNA or RNA into small discrete sequences, for which an identical sequence exists on the chip. If the sample nucleic acid finds its match, it binds and emits an electronic signal [45]. Such a human-response chip can be used to determine a patient's response to potentially toxic antimicrobial treatments in advance of delivery. If the patient lacks enzymes to break down toxins or cannot metabolize a drug effectively, alternative therapies can be used to avoid dangerous adverse effects [46]. In another prospective microarray-based test, human leukocyte response to various infectious agents may be used to pinpoint the etiology before the agent itself can be detected [47]. Development of this sort of technology is on a fast track, to facilitate detection of bioterrorism agents before the symptomatic phase of disease renders widespread preventive measurements inadequate [48]. Proteomics is being used to fine-tune the development of new anti-infectives as well [49]. One aspect of microarray technol-

ogy yet to be standardized and codified is the monumental problem of data analysis [50]. An entire new discipline of bioinformatics has developed for this purpose. This challenge of interpreting the vast volume of data points generated by microarray studies is acquired along with the technological advances, and its resolution will require a collective and extensive effort.

In summary, the future potential utility of these incredible technologies—and of numerous others not mentioned here—will revolutionize infectious diseases diagnostics. However, the shortcomings of these technologies must be recognized. Present dangers include failure to support the need for traditionally trained microbiologists, allowance of expertise to be moved to sites distant from patient care activities, inappropriate trust of new technology, and underestimation of the value of clinical diagnosis based on the acumen of experienced laboratorians and infectious diseases practitioners.

Acknowledgments

Potential conflicts of interest. E.J.B. has been on the speakers' bureau for Becton Dickinson and Cepheid and has received research support materials from Cepheid, Roche Molecular Diagnostics, and AdvanDx.

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