TB: Keeping an ancient killer at bay

By James W. Brown, PhD, HCLD

"Lungs" by Kristen Visser, at age 7, St. Mary School

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LEARNING OBJECTIVES

Upon completion of this article, the reader will be able to:

1. Describe multidrug-resistant tuberculosis and why eliminating tuberculosis in the United States is a global effort.
2. List the pros and cons of the two methods used routinely in TB identification.
3. Discuss the trends in TB-susceptibility testing.
4. Describe how TB genotyping can aid epidemiologists in tracking cases in a suspected outbreak.
5. Recognize the biosafety issues associated with working with *Mycobacterium tuberculosis,* and discuss some measures that can be taken to reduce the risk of becoming occupationally infected.

Laboratorians have been on the front lines in the fight against tuberculosis (TB) ever since Dr. Robert Koch announced his discovery of the tuberculosis bacillus in 1882. The bacillus *Mycobacterium tuberculosis* (*M tuberculosis*) has a high concentration of lipids in the cell wall, which makes it the ultimate survivor. It is impermeable to stains and dyes, resistant to many antibiotics, resistant to killing by acidic and alkaline compounds, resistant to osmotic lysis via complement deposition, and resistant to lethal oxidations and survival inside of macrophages. The bacillus also grows slowly compared to many of the other pathogenic bacteria; *M tuberculosis* divides every 16 to 20 hours as compared to *Escherichia coli*, which can divide every 20 minutes. This has been a major problem historically in identifying the organism because weeks, and sometimes months, were required to complete the "culture and susceptibility" laboratory testing.

"Ebola with wings"

According to Dr. Lee Reichman, executive director of the New Jersey Medical School National Tuberculosis Center and co-author of *Time Bomb: The Global Epidemic of Multi-Drug-Resistant Tuberculosis*, "In an era of concern over bioterrorism, it is important to remember that the disease of mass destruction is tuberculosis. According to the World Health Organization (WHO), over 2 million people died in 2003. Putting this in perspective, last year there were 813 deaths from SARS, 244 from Ebola, and five from anthrax in 2001; one cow died from mad cow disease; and there were no deaths at all due to smallpox. Paradoxically, TB is 100% preventable and curable."

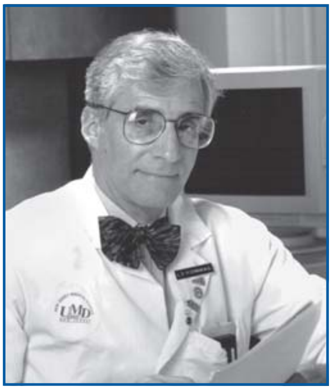

Photo courtesy of UMDNJ

Dr. Lee Reichman, international expert on MDR-TB.

Of the world's 6 billion people, 2 billion have latent TB. *M tuberculosis* infects many individuals, yet causes disease in relatively few. After the bacillus enters the lungs, the cellular arm of the immune system cannot kill the organism completely. It can, however, usually isolate it safely in the lungs. The bacteria frequently live inconspicuously for years or decades. During this latent infection, the victim generally suffers no obvious disease symptoms and cannot pass on the germ to others. Notwithstanding, if the immune system weakens (usually from old age, poor nutrition, or other diseases), the live bacteria can emerge from their hiding places and cause active TB.

While ordinary TB can be treated in a developing country with antimicrobials that cost roughly $10, sadly, TB has now returned as the leading infectious killer worldwide. "In an era of high tech, it is ludicrous that we have not had a new TB drug in 35 years," comments Dr. Reichman, who was recently in Romania assessing the global outbreak of multidrug-resistant tuberculosis (MDR-TB).

MDR-TB is defined by its resistance to isoniazid and rifampin. A recent global review showed that the prevalence of MDR-TB is 3.2% among new TB cases. The mechanism of resistance is man-made and reflects the problem of incomplete therapy. "When the HIV epidemic hits the pool of latent TB infections, there is going to be an explosion of MDR-TB. TB and HIV are like gasoline and a match," says Dr. Reichman. HIV patients, who have diminished cellular immunity, have little defense against the bacteria and can easily infect others with TB, which is spread through the air like the common cold. TB has been referred to by Richard Bumgarner, former deputy director of the WHO's global TB program, as "Ebola with wings."

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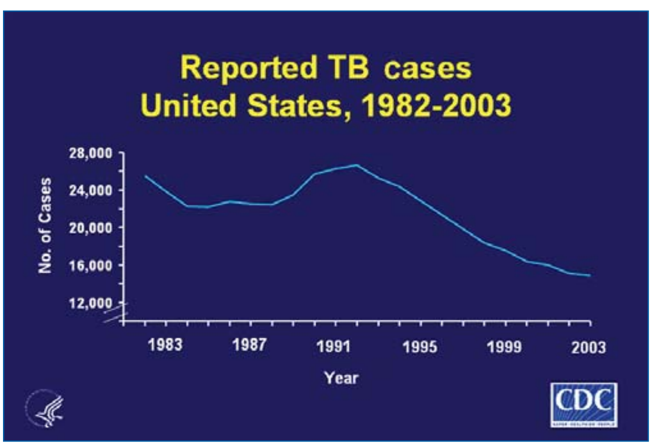

Chart from CDC 2004

Figure 1. Reported TB Cases, United States, 1982-2003. The resurgence of TB in the mid-1980s was marked by several years of increasing case counts followed by an even steeper rise. The total number of TB cases peaked in 1992. From 1992 until 2002, the total number of TB cases decreased 5% to 7% annually, and 2003 marked the 11th year of decline in the total number of TB cases reported in the United States since the peak of the resurgence.

Ending neglect

After antituberculosis drugs became widely available in the 1950s, tuberculosis began to recede from public consciousness. Since the 1960s, it has been largely ignored as a public health threat. Funding was cut and programs were closed until the killer returned in the mid-1980s, along with the first treatment-resistant strains. The subsequent increase in TB-control activities brought the number of new and existing cases in the United States to an all-time low.

In May 2000, the Institute of Medicine report, "Ending Neglect: The Elimination of Tuberculosis in the United States," warned another cycle of resurgence was likely if the United States failed to take decisive action to eliminate TB (see Figure 1). The report offered a plan detailing a number of intertwined steps involving all levels of government and the private sector, and identified clinical laboratory and TB testing as major areas of focus. The U.S. elimination goal was less than one case per million population by the target year of 2010.

TB: An imported disease

TB has become an imported disease. In 2001, the proportion of TB cases among foreign-born persons living in the United States reached 50%. Most U.S. cases are occurring in persons from Asian, Latin American, and African countries, where TB rates are five to 30 times those of the United States. Since nearly one-third of the world's population is infected with the organism, TB elimination in the United States is not possible without a substantial reduction in the global burden of TB. Dr. Reichman notes that 500 million international travelers passing through 5,000 international airports each year increases the risk for everyone. "To control TB anywhere, you have got to control it everywhere."

The future of TB laboratory services

The Association of Public Health Laboratories (APHL) just published a new report entitled "The Future of TB Laboratory Services: A Framework for Integration, Collaboration and Leadership," which says, "The critical next step will be to develop an integrated system that ensures timely laboratory testing and timely flow of information among laboratorians, clinicians, and TB controllers." John Ridderhof, Dr PH, chief, Office of Laboratory Systems Development Division of Laboratory Systems, Public Health Practice Program Office, at the Centers for Disease Control and Prevention (CDC), gives numerous suggestions as to how laboratories can better work together to speed up the process and provide better turnaround times. For example, if a laboratory has already identified *M tuberculosis* from a specimen, the next specimen could be sent to a full-service laboratory to avoid delay in reporting back to the clinician and TB-control personnel, and to reduce delay in treatment.

Another tip is to send broth cultures along with slants. Subculture from slants can cause delays of several days. Dr. Ridderhof states that laboratories should concentrate on the already existing CDC guidelines[1] for acid-fast bacillus (AFB) smear, culture, and drug-susceptibility testing. For instance, does the laboratory receive specimens for TB testing within one day of specimen collection? Are smear results reported to a patient's provider within one day of specimen receipt? Are results reported to the local health department within one working day from the

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time they are reported to the specimen submitter? A recent report[2] showed that in California, laboratory reporting to the specimen submitter was delayed for 26.9% of smear-positive patients and 46.8% of smear-negative patients.

A visit to a hospital network

Susan Whittier, PhD, D(ABMM), chief of Clinical Microbiology Service at Meridian Health Systems in Neptune, NJ, oversees mycobacteriology testing for the entire hospital system. "We are really very fortunate since our microbiology laboratory at Jersey Shore University Medical Center

Photo courtesy of Dr. Jim Brown

Rick Valdez, MT (AMT), and Dr. Susan Whittier in Mycobacteriology Laboratory at Jersey Shore University Medical Center.

serves as the core lab for our hospital system. We consolidated three very active and diverse labs and have been able to reap numerous benefits. We function as a Level-3 lab, and we are able to definitively identify over 90% of our isolates. We use the Gen-Probe Accuprobe system to identify *M tuberculosis*, *M avium* complex, *M gordonae*, and *M kansasii*. All others are sent to our reference laboratory — ARUP in

Photo courtesy of Dr. Jim Brown

Rick Valdez, MT (AMT), manipulates TB cultures while working safely in a Biological Safety Cabinet with gloves, gown, and disposable respirator.

Salt Lake City. Since our core lab is just a few miles from the beach, a frequent isolate we send for identification is *M marinum*. In general, our level of service is perfectly matched to the patients we encounter.

"Even though we are not in a metropolitan area, we see our share of tuberculosis cases. We serve patients who have emigrated from endemic regions throughout the world, and our beaches attract many people from New York City during the summer. All of our hospitals 'think TB.' When a patient presents to our emergency department at 3 o'clock in the morning, appropriate airborne precautions are put into place immediately. We process samples once a day, seven days a week. 'Rule-out' TB patients remain in isolation until we are able to turn around three negative AFB smears. When we do have a positive smear or culture, we notify the physician, the floor, the infection-control practitioner, and our state department of health. We do not wait for growth on solid media to run our probes; we will always try to expedite results by testing the broth as soon as it is flagged as positive for AFB."

Photo courtesy of ARUP Laboratories

ARUP Mycobacteriology Group. L to R (front), Gail L. Woods, MD, Haleina Neal. L to R (back), Joshua Kunz, Geremy Smith, and Joseph Strasburg.

TB testing at the large commercial laboratories and large medical centers

Gail L. Woods, MD, is the medical director of ARUP's Specialty Microbiology and Microbial Antigen Detection Laboratories, which serve clients in all 50 states. The large commercial laboratories, such as LabCorp, Quest Diagnostics, and ARUP, provide excellent systems that bring specimens to the laboratory in a timely fashion and that employ electronic reporting capabilities. Dr. Woods states that to get specimens into the laboratory from all across the United States takes approximately 24 hours, with smear results having a 15-hour-or-less turnaround time. ARUP phones the referring laboratory with any positive TB results and directly reports any positives originating in Utah to that state's TB-control program. Client laboratories are then directed to notify their own local or state health departments. It is the client laboratory's responsibility to notify the clinician treating the patient and the TB-control program in the state where the TB patient resides. This may be a weak link in the chain.

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Photo courtesy of Dr. Jim Brown

Pennsylvania Department of Health, Bureau of Laboratories, Microbiology Group L to R, Nancy G. Warren, PhD, assistant director; Gisela S. Withers, Microbiologist 3, Supervisor, Bacteriology Section; Ismail Dirie, Alfred Logan, Melanie Solomon, Donna Krouse, and William Sweimler, all Microbiologist 2 level; Wayne Chmielecki, Microbiologist 3, Supervisor, Molecular Microbiology Section; and Bruce Kleger, Dr PH, director.

Dr. Woods believes that the future holds more molecular-based testing and less biochemistry; that probes work well and have helped accelerate identification; and that susceptibility testing will utilize short-incubation liquid-based systems.

A visit to Pennsylvania's Bureau of Laboratories

Bruce Kleger, Dr PH, the director, and Nancy G. Warren, PhD, the assistant director of the Pennsylvania Department of Health, Bureau of Laboratories in Lionville, PA, explain the essential role of public health laboratories in TB testing. "Public health laboratories have always been at the forefront of TB testing — it is one of the core public health functions that our laboratory supports. Common to all clinical, commercial, and public health laboratories is the potential for TB testing. Typically, all three may perform primary detection methods, such as smear, culture, and even PCR, but it is usually public health and the larger reference laboratories that confirm identification, speciate other mycobacteria, and perform drug-susceptibility testing," says Kleger. "Usually, public health laboratories facilitate and provide confirmatory and essential epidemiology support through expanded drug-susceptibility testing and genotyping. Communications remain a strong requirement of any successful control program. Any laboratory performing TB testing should be vigilant in reporting positive findings to its state TB-control program. When TB reporting fails, control measures will not be implemented, and the cycle of disease remains unchecked."

TB spreads through the air like the common cold and has been referred to as "Ebola with wings."

Dr. Kleger relates that, during the TB resurgence of the 1990s, the laboratory accepted more specimens for testing and enhanced working relationships with state TB-control and HIV program staff. Additionally, revisions to the state's communicable disease law now require that TB isolates be submitted by clinical labs to provide the opportunity for uniform culture confirmation and drug-susceptibility testing.

Current methodologies for identification of TB

According to Dr. Warren, the laboratory scientists at Pennsylvania's Bureau of Laboratories explain that cultures of *M tuberculosis* are routinely identified by biochemical and nucleic acid probes. Biochemical testing is the more familiar, conventional approach; *M tuberculosis* produces niacin, while most other mycobacteria do not have this ability. Nucleic acid probes that detect TB ribosomal RNA have been in use for some time now. These probes are a dependable method for culture identification and eliminate the need to handle the chemicals used in biochemical testing. Both methods have pros and cons. Conventional testing definitely identifies *M tuberculosis*, but cultures must be several weeks old before the tests can be performed. Probes can be performed much earlier in the culture process, can provide answers within two hours, and can be combined with liquid culture methods. Probes are not able to separately identify all members of the "tuberculosis complex," because probes will yield positive results when tested against *M tuberculosis*, *Mycobacterium bovis*, *bacillus Calmette-Guerin*, *Mycobacterium africanum*, and *Mycobacterium microti*. Overall, the rapid turnaround time achievable with probes generally far outweighs the need to subdivide the tuberculosis complex; if this level of identification is needed, probe positive isolates can be sent to a reference lab.

Current methodologies for TB-susceptibility testing

Currently, drug-susceptibility testing (DST) is performed by two general methods. Conventional methods use solid media, such as the Middlebrook agar used in the United States, with drugs incorporated into the agar. DST in liquid media is another accepted method and provides a much faster turnaround time (one week vs. three weeks). Liquid-media DST also can be supported by instrumentation — making reading, incubating, and interpreting results less laborious. DST is often sent to public health laboratories. In those laboratories that perform DST, liquid-media testing is quite common and isolates are usually not tested by solid-media methods unless a question arises or drug resistance is detected and results confirmation is needed.

New trends in TB identification and susceptibility testing

Dr. Kleger and Dr. Warren explain that new developments in recent years are having an impact on TB testing. The need for faster identification of new cases of TB has led to a real push for faster lab testing. Amplified DNA assays are now available to detect TB directly in respiratory secretions, so waiting for culture is not as critical. Culture and susceptibility testing have been improved by semiautomated broth methods, but the area of identification is where the most recent improvements have been seen. Methods have been developed that provide identification by high-performance liquid chromatography and 16S sequencing. In comparison to conventional methods, results are obtained faster, more species can be detected, and testing is better controlled. The combination of liquid culture and new identification methods has helped us identify many new mycobacterial species, and more species may be detected in the future.

Photo courtesy of Dr. Jim Brown

Donna Krouse, MT(ASCP), analyzing mycolic acids by high-performance liquid chromatography (HPLC) to identify *M tuberculosis* at the Pennsylvania Department of Health, Bureau of Laboratories.

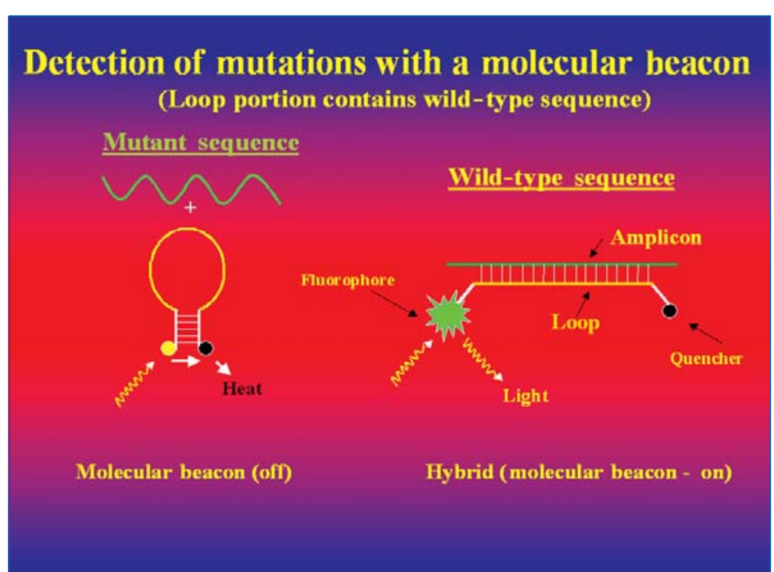
DST is on the cusp of new discovery right now. Liquid-media DST has certainly been a time improvement over conventional solid media, but neither provides the most rapid path to detecting drug resistance. As a result of the 1990s TB resurgence, more attention is now focused on the problem of dealing with drug-resistant and multidrug-resistant organisms. Improving the time it takes for lab tests to detect drug resistance is a step towards managing the complicated disease, and reports in the scientific literature today point to efforts to do just that. Amplified probe methods and molecular beacons are just two of the new tests being evaluated. In the next few years, laboratorians can anticipate that these tests will move out of the research lab and into the clinical lab for routine use.

Molecular beacons

Edward Desmond, PhD, of the Microbial Diseases Laboratory Branch of the California Department of Health Services, describes the use of molecular beacons. Longstanding guidelines from the CDC and the APHL have given laboratories a time frame of two to four weeks to detect the presence of *M tuberculosis* in a culture and determine whether the strain is susceptible or resistant to commonly used anti-TB drugs, such as isoniazid, rifampin, and ethambutol. This requires growing a culture of these slow-growing organisms twice — once for initial isolation and once in the presence and absence of the drugs to determine susceptibility. In many cases, this long turnaround time has no detrimental effects if patients are put on standard therapy before laboratory results are available and if their infecting organisms are susceptible to the standard therapy. With an initial treatment regimen of isoniazid, rifampin, ethambutol, and pyrazinamide, and the prevalence of resistance to isoniazid close to 10% and to rifampin at around 1% to 2%, the standard regimen will usually treat tuberculosis successfully or at least keep the infection in check until the drug-susceptibility results are available. In some cases, however, this conventional turnaround time is not nearly fast enough.

Quick drug-susceptibility results are always better in order to guide the TB patient's treatment regimen, help him to get better quickly, and reduce further spread of TB. Sometimes, quick results are especially important, however, when a culture-positive patient — for example, a healthcare worker, teacher, or child-care worker — has exposed a susceptible group of people to TB. Some of the exposed people may show signs of infection, like a positive skin test. TB-control guidelines call for treatment of infected people with a preventive drug regimen, such as isoniazid. If the culture-positive patient who exposed others has an isoniazid-resistant strain, preventive therapy with this drug will be ineffective; another preventive regimen or strategy will need to be chosen.

Dr. Desmond explains that another situation in which it is imperative to obtain the quickest possible drug-susceptibility result is when a critically ill patient, i.e., a patient with another illness like AIDS or cancer, contracts TB. If the patient has drug-resistant TB, and the initial regimen is ineffective, the patient could die or become seriously worse before the two- to four-week turnaround time of the conventional culture-based methods. On the other hand, blind treatment of such a patient's TB with second-line drugs would entail the greater toxic effects of these drugs.



Courtesy of Dr. W. Prober

Figure 2

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These clinical situations provide a setting for selective use of rapid molecular testing to detect drug resistance. One such method is a real-time PCR assay using molecular beacons.[3] Molecular beacons are DNA probes designed with a structure that lets them "light up" in a real-time PCR reaction if there is amplification of their specific target (see Figure 1). The probe or loop region of the molecular beacon will bind to the normal DNA sequence of an *M tuberculosis* gene, such as catalase (*kat*G) or RNA polymerase (rpoB), separating the fluorescent moiety from the quencher moiety of the beacon, resulting in fluorescence. Mutations in these genes have been associated with resistance to isoniazid and rifampin, respectively, the most effective drugs commonly used to treat TB. In a PCR reaction in which selected segments of the *kat*G or rpoB gene are amplified, molecular beacons can discriminate between the presence of the wild-type sequence associated with drug susceptibility or a mutated sequence at specific sites associated with drug resistance. Figure 2 shows the output of a molecular beacons experiment, looking for mutations in the *kat*G gene associated with isoniazid resistance. The nonmutated strains will show a rise in fluorescence with time as the molecular beacon probes bind to their complementary sequence in the amplified product, separating the fluorescent moiety from the quencher. Resistant strains with mutations in the probe region will not bind to the molecular beacons, so their fluorescence pattern stays flat throughout the amplification.

Commercial test kits are not available at present for performing molecular beacons testing to determine TB drug-susceptibility or resistance. Dr. Desmond predicts that because molecular beacons are so quick and generally provide clear-cut results, their use in the future will likely become more widespread. For phenotypic, culture-based testing, these methods — besides being slow — involve the need for rigorous control of growth-medium composition, inoculum size, and metabolic status of the inoculum to permit accurate results to be obtained. These factors, in the long run, may make molecular testing more practical than phenotypic testing for most laboratories.

500 million international travelers passing through 5,000 international airports each year increases the risk for everyone.

TB genotyping

Dr. Desmond highlighted other tools created in the laboratory for TB epidemiology. Before the development of molecular strain typing of tuberculosis in the 1990s, tuberculosis-control personnel often relied on interviewing patients and looking for TB cases in the patients' close contacts — people who shared social or work settings or living facilities — to trace the spread of this disease. A recent study has shown that strain typing, with follow-up interviews for cases with the same strain type, can expand the understanding of transmission, including in settings outside the traditional concentric-circle approach.[4] Genotyping, thus, has the potential for identifying new circumstances and places where TB transmission can occur.

New PCR-based genotyping methods, including spacer oligonucleotide typing (spoligotyping) and mycobacterial interspersed repetitive units (MIRU) typing, can provide strain-

Photo courtesy of California Department of Health Services

Francine Arroyo works on MIRU with capillary sequencer.

typing information from cultures within a month or sometimes less from the time the specimen is collected from a patient. The CDC has contracted with two laboratories (Michigan State Laboratory for the eastern half of the United States and the California [Microbial Diseases] Laboratory for the western half) to provide genotyping services for all culture-positive U.S. patients. These laboratories accept pure broth or solid-medium cultures of *M tuberculosis* complex organisms submitted through participating state laboratories. The Michigan and California laboratories use MIRU and spoligotyping, and report results within two weeks of receiving the culture in most instances. Reports, including a cumulative database, are sent to the tuberculosis-control program in each state. Universal genotyping with rapid results offers the possibility of new knowledge and rapid response to developing outbreaks of TB.

TB genotyping has been useful in answering specific questions related to suspected outbreaks of TB in institutions, such as hospitals, schools, and prisons. In California, for example, a renal-dialysis clinic reported five cases of tuberculosis in a year's time. It was suspected that transmission of TB was taking place at the clinic and that more rigorous control measures would need to be implemented. TB genotyping showed, however, that the five patients had different strains so that transmission at the clinic was effectively ruled out. Conversely, after an outbreak in a prison was thought to be under control, a new case was found to be the outbreak strain again, leading to further control measures.

When used together for strain typing, spoligotyping and MIRU provide a powerful combination of tools that can discriminate strains which are not part of the same chain of transmission or the same cross-contamination event. Occasionally, however, unrelated strains could have the same spoligotyping and MIRU numbers. For this reason, a third technique, insertion sequence 6110 restriction fragment length polymorphism (RFLP) is available in the regional laboratories. When it is suspected that cultures from patients with matching spoligotyping and MIRU numbers may be unre-

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lated epidemiologically, TB-control programs can request that RFLP testing be done as well. RFLP is a lengthier and more labor-intensive method than the PCR-based methods, so about three weeks is required after RFLP is requested before results are available.

The CDC and the National Tuberculosis Controllers Association prepared a Genotyping Guide — 80 pages long — which is available at *http://web-tb.forum.cdc.gov*. After registering for this website, users can access the Genotyping Guide, which includes an introduction to the methods and case studies, along with information on how to access, interpret, and use genotyping services. Rapid TB genotyping by regional laboratories will unfold new possibilities for improving tuberculosis control. TB-control programs can rapidly discover patterns of transmission in developing outbreaks, and new situations in which TB is spread from one person to another are likely to be found by universal application of these techniques.

A view of the future from a pioneer in rapid TB testing

Becton Dickinson (BD) Fellow Salman H. Siddiqi, PhD, is considered the "father" of the first rapid semiautomated liquid-medium-based TB-culture system, which he helped develop at the University of Maryland. Historically, there had been no new significant development to improve laboratory testing for TB until 1980 when BD introduced the BACTEC 460 TB Radiometric System. This technology led to a shortened turnaround time for detection and drug-susceptibility testing of TB from months to weeks. BACTEC 460 became a gold standard worldwide and the workhorse of the mycobacteriology laboratory. The new nonradiometric BACTEC MGIT 960 System is a fully automatic system and, thus, has helped not only standardize testing but has also helped save labor costs. While a liquid-culture system has replaced conventional solid media in developed countries, culture-based diagnosis in general, and rapid liquid-culture systems in particular are being adopted as the resources are more readily available in developing countries.

Photo courtesy of BD

BD's "father of rapid TB testing," Dr. Salman Siddiqi, is shown with a row of the "workhorse" BACTEC 460 instruments in the background with the new BACTEC MGIT 960 in the front.

According to Dr. Siddiqi, the National Committee for Laboratory Standards (NCCLS) has placed streptomycin in the second line of anti-TB drugs (NCCLS, M24-A). This decision will not have any significant impact on BACTEC 460 or BACTEC MGIT 960 testing because both systems have the ability to test five drugs for susceptibility, and any drug combination may be tested on these instruments. Most overseas laboratories, as well as many laboratories in the United States, are still testing streptomycin. As medical and laboratory practices evolve in the next few years, the packaging for the drug combination will also evolve accordingly.

Microarray-chip technologies

Dr. Siddiqi says that he sees the new trends are toward achieving faster and faster results. In developed countries, molecular technologies are further reducing testing time from weeks to days or even hours. Results of molecular tests, however, are still used in conjunction with the conventional culture-based techniques. Microarray-chip technologies are developing quickly, and this new approach may one day also become a gold standard in carrying out multiple TB testing on a single chip and would have a great impact on the management and control of the disease. The concern is that these new state-of-the-art technologies are complex and expensive and are not suitable for low-resource developing countries where 95% of the global burden of tuberculosis is located. In many of these countries, AFB smear is the only test that can be afforded. It may take decades before newer technologies become widely accessible in these countries. At present the culture-based techniques may be the only hope for diagnosis of the disease in high-burden developing countries.

Biosafety: OSHA withdraws the proposed new TB standard

Laboratorians working with *M tuberculosis* have always been at risk for becoming occupationally infected with the organism. After a decade of intense debate, on December 31, 2003, the Occupational Safety and Health Administration (OSHA) withdrew the proposed new TB standard and also eliminated the interim TB standard 29 CFR 1910.139. An Institute of Medicine report entitled "Tuberculosis in the Workplace" warned that the OSHA standard may not allow organizations reasonable flexibility to adopt TB-control measures appropriate to the level of risk that workers face. Many other infection-control groups, including the Society for Healthcare Epidemiology of America and the Association for Professionals in Infection Control and Epidemiology (APIC), agreed with that assessment. OSHA decided that a number of factors have emerged in the past decade that alleviate the necessity of developing a TB-specific regulation. For example, there has been a steady decrease in the number of TB cases nationwide. OSHA determined that occupational risk was lower than originally reflected because of greater implementation of TB controls and greater compliance with the CDC's guidelines. It concluded that a new rule would not substantially reduce the spread of TB from undiagnosed sources.

OSHA has introduced a new mandate applying the General Industry Respiratory Protection Standard to *M tuberculosis*.[5] This mandate became effective July 1, 2004, and requires the annual fit-testing of respirators in healthcare settings. Many infection-control groups have been working diligently to overturn this new ruling. APIC has long opposed the notion of mandatory annual fit-testing, because its membership believes there is no solid scientific justification for this practice. Jeanne Pfeiffer, RN, MPH, CIC, the current president of APIC, responded to an August 15, 2004, *Washington Post* article on the issue, "rather than mandating unproven, unreliable, and unnecessary ritualistic practices such as annual fit-testing, the federal government should instead focus on ensuring dedicated funding for TB-control efforts at the public health level — efforts that are proven to be effective." The jury is still out on the validity of respirator fit-testing and TB.

The authoritative standard for biosafety in the clinical laboratory remains the *Biosafety in Microbiological and Biomedical Laboratories (BMBL) 4th Edition* published by the CDC and the National Institutes of Health in 1999. Freely available on the Internet at *www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm*, the book contains helpful and practical information about how to work safely with *M tuberculosis*, warns that all aerosol-generating activities must be conducted in a Class I or II biological safety cabinet, and recommends the use of a slide-warming tray, rather than flame-drying, for slides. The incidence of tuberculosis in laboratory personnel working with *M tuberculosis* was three times higher than that of those not working with the agent before workers focused on biosafety issues.

Making decisions about TB testing

Clinical laboratories today must decide how they will handle requests for TB tests. If testing is to stay in-house, then what extent of testing will be performed? Will an AFB smear result provide the physician with immediate information needed? Is it necessary to perform culture in-house, or can this be referred? What identification methods should be used to provide the fastest answers?

With personnel costs and unique instrumentation requirements, the best choice may be to refer specimens to another laboratory. Choose your reference laboratory carefully, and ask critical questions, such as:

- Are instructions provided for specimen collection, storage, and transport?
- What are the criteria for rejecting a specimen?
- How is information communicated back to the submitting laboratory?
- Does the reference laboratory participate in an approved proficiency-testing program?
- How does the reference laboratory maintain proficiency in performing requested mycobacteriology tests?
- What testing methods are used?
- What are the expected turnaround times?
- How are positive cultures stored and retained?
- Is there a disaster plan in case testing cannot be done? ☐

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James W. Brown, PhD, HCLD, and dean of the division of Health Sciences and Human Performance at Ocean County College in Toms River, NJ, is pictured here in Guatemala in September 2004 with his newly adopted daughter, Abby Carmelina. Many internationally adopted children have positive Mantoux tests, some of which are due to infection with *M tuberculosis*.

Photo courtesy of Sally Jacober-Brown