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Recent advances in the molecular diagnosis of tuberculosis

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To date, the diagnosis of tuberculosis has not improved significantly and still relies heavily on staining and culture of sputum or other clinical specimens which were developed more than 100 years ago. Staining does not differentiate tuberculosis from other mycobacterial infections, and culture requires at least 4 to 8 weeks. These are the major problems faced by tuberculosis control programs. In response to this demand, new rapid diagnostic methods are urgently sought. In recent years, much hope has been laid on the development of molecular techniques in the routine tuberculosis laboratory. This review concentrates on 4 techniques that are increasingly used in clinical laboratories: polymerase chain reaction to detect mycobacterial DNA in clinical specimens, nucleic acid probes to identify culture, restriction fragment length polymorphism analysis to compare strains for epidemiologic purposes, and genetic-base susceptibility testing methods for rapid detection of drug resistance. Finally, the increase in the use of clinically-useful molecular biological techniques that affect turnaround time, length of stay, and patient outcome, and reduce overall hospitalization costs will continue until universal standardization for molecular diagnostic procedures are provided. At present, conventional methods should not be replaced by novel methods until the latter are shown to be of equal or greater sensitivity, specificity, reliability, and user-friendliness. However, it is expected that the newly developed molecular techniques will complement our armamentarium of diagnostic tools in the detection of tuberculosis. It is also expected that clinical protocols based on molecular methods will increase the chances for cure by selecting the most appropriate treatment and improving the quality of life of tuberculosis patients.

Key words: Molecular diagnosis, tuberculosis, nucleic acid amplification, nucleic acid hybridization, polymerase chain reaction, restriction fragment length polymorphism

Mycobacterium tuberculosis is an important human pathogen, responsible for 2 billion people worldwide with latent infection, 8 million new cases a year, and up to 3 million annual deaths [1]. In the past decade, the increase in the prevalence of tuberculosis (TB), the emergence of multidrug-resistant strains, and the explosive interaction between *M. tuberculosis* and the human immunodeficiency virus have created public health urgency for early identification of *M. tuberculosis* infectious individuals and initiation of appropriate anti-TB chemotherapy [1-3]. Direct demonstration of acid-fast bacilli (AFB) in clinical specimens by Zeihl-Neelsen stain and isolation of *M. tuberculosis* by the conventional culture method, which were developed in the late 1800s and are the oldest tests still in clinical use, are considered gold standard for the laboratory diagnosis of TB infection [4]. Despite the long history of clinical application, limitations and controversy with regard to sensitivity and interpretation remain [5,6]. Significant effort is being made in the development of new techniques for the diagnosis of TB in order to overcome the limitations of currently available diagnostic methods. In the past 10 years, progress in molecular biology created new opportunities for development of new methods with an attempt to achieve the shortest turnaround time for clinical laboratory diagnosis [7,8]. In light of these findings, the use of molecular techniques for the laboratory diagnosis of *M. tuberculosis* and their clinical applications are reviewed.

Polymerase Chain Reaction

Amplification techniques, namely, the polymerase chain reaction (PCR) and its derivatives have attracted considerable interest as a diagnostic tool for TB, particularly with the hope of shortening the time required to detect and identify *M. tuberculosis* in respiratory specimens [8]. With the use of PCR, nucleic acid sequences unique to *M. tuberculosis* can be detected directly in clinical specimens, offering better

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accuracy than AFB smear and greater speed than culture [9-11].

The PCR provides a means of amplifying DNA *in vitro* by the addition of the DNA polymerase and ribonucleotides and DNA specific for *M. tuberculosis* (primers). The amplification cycle is repeated many times, and in a short period millions of DNA fragments are readily detectable. Polymerase chain reaction has been used to detect *M. tuberculosis* in respiratory samples and other clinical samples. However, because laboratories that use PCR techniques have produced their own tests with a wide variety of primers, probes, methods of DNA extraction, PCR protocols, and detection methods, the clinical sensitivity of PCR compared with that of culture has been reported with wide variations [12]. These drawbacks may be improved by use of a commercially-available PCR kit, which has the potential for better overall performance characteristics in clinical laboratories. To date, 2 systems have been approved by the United States Food and Drug Administration (FDA) for use on smear-positive respiratory specimens: the Amplified Mycobacterium Tuberculosis Direct Test (MTD) (Gen-Probe, San Diego, CA, US) and the AMPLICOR *M. tuberculosis* Test (Roche Diagnostic Systems, Indianapolis, IN, US). Numerous studies have confirmed a sensitivity and specificity well over 95% in smear-positive respiratory specimens, but were much lower when testing smear-negative samples [13-15]. Recently, Gen-Probe modified the MTD with the aim of increasing sensitivity and decreasing turnaround time. The FDA approved this enhanced MTD in September 1999 for testing respiratory specimens, regardless of the smear result [16]. The experience in using commercial kits in Taiwan is limited. One report from Huang *et al* [17] found that the AMPLICOR kit had a sensitivity, specificity, positive predictive value, and negative predictive value of 73.81%, 97.18%, 93.94%, and 86.25%, respectively. Unfortunately, the performance of both tests on extrapulmonary specimens has varied [18,19], and neither of the manufacturers make claim that nonrespiratory specimens can be tested with their assay. To facilitate clinical application, laboratories must use in-house PCR for detection of extrapulmonary TB. Among the numerous amplification assays, IS*6110*-based methodologies become the most commonly used in-house PCR method in the clinical mycobacteriology laboratory [20]. IS*6110*, which belonging to the IS3 family, is found in virtually all members of the *M. tuberculosis* complex and is apparently restricted to this group of organisms [21,22]. We found evidence of IS*6110*-based methodologies in respiratory samples from patients in the Taipei Veterans General Hospital, Taiwan. The sensitivity and specificity compared with culture were 94.7% and 100%, respectively, and 76.7% and 98.6% for the smear-negative and culture-positive samples, respectively [23]. The overall sensitivity, specificity, positive predictive values, and negative predictive values compared with culture results were 91.7%, 98.6%, 98.8%, and 90.6%, respectively [23]. Although the high sensitivity and specificity of the in-house PCR is encouraging, there is a well-recognized interlaboratory variation in the results using PCR to detect the IS*6110* sequence [12]. At the current state of development, implementation of this novel method should take into account the performance of laboratories, the local epidemiologic situation, and should be based on cost-effective analysis [24].

Nucleic Acid Probe Methods

Nucleic acid probe tests are gaining importance in the identification of mycobacteria since the beginning of the 1990s when commercially-available nonradioactive (chemiluminescent) probes (Accuprobe; Gen-Probe, San Diego, CA, US) were introduced for rapidly identifying cultures at the species level. The use of nucleic acid probes for rapid identification of mycobacteria and maximizing cost effectiveness has been extensively reviewed [25,26]. Currently, 2 probe systems are available commercially. The Gen-Probe system uses a labeled DNA probe complementary to the ribosomal RNA in *M. tuberculosis*. Another available probe is designated SNAP (Syngene, San Diego, CA, US), which uses a probe labeled with alkaline phosphatase that is directed against ribosomal RNA. Originally, these probes were designed for culture confirmation of organisms, perhaps 10^5 organisms per sample, in a liquid or solid medium and are 99% to 100% specific [24,27]. Probes are also available for *Mycobacterium avium*, *Mycobacterium intracellulare*, *Mycobacterium avium* complex, *Mycobacterium gordonae*, and *Mycobacterium kansasii* [24]. An additional advantage of these probes is the shelf life of 6 to 12 months. Therefore, the nucleic acid probes alone or in combination with other identification methods are used widely in routine clinical practice, which serve as a substitute for biochemical testing to identify mycobacterial species and are more accurate than biochemical methods. The only drawback reported is the detection limits of approximately 105 organisms per reaction tube, which will require to culture the specimens long enough to get such a large number of organisms.

Molecular Typing of *M. tuberculosis*

Key factors in the fight against TB are rapid detection, adequate therapy, and contact tracing to arrest further transmission. Recent developments in molecular technologies have led to methods for rapid tracing of TB transmission routes by differentiating clinical isolates based on polymorphism in genomic DNA of *M. tuberculosis* [28]. Furthermore, DNA fingerprinting of *M. tuberculosis* provides a versatile tool for the identification of transmission [29], investigation of TB outbreaks [30,31], distinction between reactivation and reinfection [32], as well as proof of laboratory cross-contamination [33,34]. The most commonly used method for genetic typing of *M. tuberculosis* complex strains is restriction fragment length polymorphism (RFLP) analysis, by Southern blotting of genomic DNA and the mobile element IS*6110* as a probe [35]. IS*6110*-based RFLP has shown a high degree of heterogeneity in typing patterns and reproducibility [36], which has become the standard method for fingerprinting of *M. tuberculosis* (Fig. 1). However, it has been hampered by the need to culture this slow-growing organism and by the level of technical sophistication needed for RFLP typing [35,37].

Other types of multicopy DNA such as direct repeat (DR) sequences [38], spacer oligotides between the DR sequences (spoligotyping) [39], and polymorphic GC-rich repetitive sequences (PGRS) [40] have been used as a secondary probe in subdividing clusters of *M. tuberculosis* with fewer IS*6110* copies [41] or in differentiating *Mycobacterium bovis* isolates from animals [29].

Genotypic Methods for Drug Susceptibility Testing

Appropriate therapeutic decisions are based on the result of anti-TB drug susceptibility testing. Traditional susceptibility tests are not only time-consuming, but have also significant technical problems in their standardization [42]. Recently developed molecular biological techniques have significantly helped in understanding the basis of drug action and resistance mechanisms in *M. tuberculosis* [43,44]. In the case of rifampicin, for example, 95% of resistance is caused by mutations in the *rpo*B gene, which codes for a subunit of the DNA-dependent RNA polymerase enzyme [45]. Molecular tests have been designed to detect mutations in *rpo*B gene responsible for rifampicin resistance [46-50]. Recently, we have successfully demonstrated that susceptibility testing of *M. tuberculosis* to rifampicin could be accomplished rapidly with acceptable accuracy by PCR-reverse dot blot (RDB) hybridization (Table 1). The results of PCR-RDB assay are available within one day, while the proportion method requires 3 weeks to complete. Similar approaches have been used to detect resistance to other anti-TB agents [51,52]. Unfortunately, resistance to anti-TB drugs involves changes in multiple genes and at multiple potential locations. In addition, not all possible genes or mechanisms of resistance have been identified. These make testing for the various genes difficult and represent a significant drawback for diagnostics. Given the complexity of genotypic methods, there has been interest in high-density probe arrays (gene chips) that would facilitate screening for mutations in an amplified product [53-55]. Research is still in progress to develop rapid tests for drug resistance that are suitable for use in the routine TB laboratory.

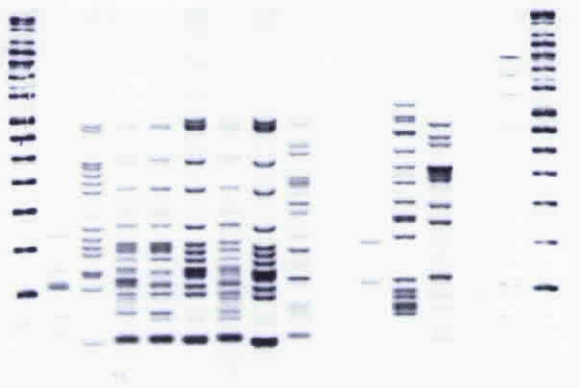

Fig. 1. IS*6110*-based DNA fingerprint patterns from isolates of *M. tuberculosis* from patients in the tuberculosis ward in Taipei Veteran General Hospital. Molecular weight markers are along both margins. The number and position of the bands varies

What is the Appropriate Use of the Rapid Diagnostic Tests for TB?

In clinical practice, the use of molecular testing for the diagnosis of TB, in combination with "classic" diagnostic tools, can greatly enhance the diagnostic ability of pulmonary clinicians, particularly in paucibacillary infections and in patients with atypical presentation, such as immunodeficient individuals. We now need to ask what is the appropriate use of the molecular diagnostic tests for TB. This question cannot yet be definitively answered, because these tests do not yet perform ideally in all circumstances. Clinicians must be careful in correlating the molecular test results with the clinical aspects of each case; sometimes multiple testing or alternative types of information are of great value. With appropriate use, they can improve the accuracy in diagnosing TB, and cut the spread of TB in the community and thus the costs of health care.

Table 1. Sensitivity and specificity of PCR-RDB hybridization in detection of rifampicin resistance from clinical isolates

PCR-RDB result	Results of proportion method		Sensitivity, %	Specificity, %
	Resistant strains	Susceptible strains		
Mutant type	33	0	100	100
Wile type	0	72		

Abbreviation: PCR-RDB = polymerase chain reaction-reverse dot blot

Conclusions

The mycobacteriology laboratory of today has a critical role in the control of TB by rapid turn-around times, allowing for the provision of optimal medical care, infection control, and public health management of TB patients. Much is expected from molecular biology techniques for the rapid, sensitive, and specific diagnosis of TB. However, the necessary resources and expertise are seldom available in clinical practice. Furthermore, quality control problems and the lack of standardization render molecular techniques unreliable in the hands of the inexperienced. After all, the performance of a good test relies on its user, and the whole medical community should not be deprived of the use of these novel rapid molecular methods because of its poor use in the hand of a few clinicians. We are only beginning to understand how to use these new tests, which cannot replace the traditional smears and cultures. It is promising that molecular technologies will take us to a new era of advanced, effective, and rapid diagnosis of TB, and in response to this, committees of experts should provide the medical community with guidelines on how to appropriately use molecular techniques for diagnosing of TB. Finally, cost-effectiveness and economic constraints also require careful consideration, as these techniques are expensive.

References

1. Bloom BR, Murray CL. Tuberculosis: commentary on a re-emergent killer. Science 1992;257:1055-64.
2. Raviglione MC, Snider DE, Kochi A. Global epidemiology of tuberculosis: morbidity and mortality of a worldwide epidemic. JAMA 1995;273:220-6.
3. Cohn DL, Bustreo F, Raviglione MC. Drug-resistant tuberculosis: review of the worldwide situation and the WHO/IUATLD global surveillance project. Clin Infect Dis 1997; 24:S121-30.
4. Schluger NW, Rom WN. Current approaches to the diagnosis of active pulmonary tuberculosis. Am J Respir Crit Care Med 1994;149:264-7.
5. Daniel TM. Rapid diagnosis of tuberculosis: laboratory techniques applicable in developing countries. Rev Infect Dis 1989;11(Suppl 2):S471-8.
6. Levy H, Feldman C, Sacho H, van der Meulen H, Kallenbach J, Koornhof H. A reevaluation of sputum microscopy and culture in the diagnosis of pulmonary tuberculosis. Chest 1989; 95:1193-7.
7. Pfaller MA. Application of new technology to the detection, identification, and antimicrobial susceptibility testing of mycobacteria. Am J Clin Pathol 1994;101:329-37.
8. Butcher PD, Hutchinson NA, Doran TJ, Dale JW. The application of molecular techniques to the diagnosis and epidemiology of mycobacterial diseases. J Appl Bacteriol 1996;81(Suppl): S53-71.
9. Eisenach KD, Sifford MD, Cave MD, Bates JH, Crawford JT. Detection of *Mycobacterium tuberculosis* in sputum samples using a polymerase chain reaction. Am Rev Respir Dis 1991;144:1160-3.
10. Crawford JT. New technologies in the diagnosis of tuberculosis. Semin Respir Infect 1994;9:62-70.
11. Jonas V, Longiaru M. Detection of *Mycobacterium tuberculosis* by molecular methods. Clin Lab Med 1997;17:119-28.
12. Noordhoek GT, Kolk AH, Bjune G, Catty D, Dale JW, Fine PE, Godfrey-Faussett P, Cho SN, Shinnick T, Svenson SB. Sensitivity and specificity of PCR for detection of *Mycobacterium tuberculosis*: a blind comparison study among seven laboratories. J Clin Microbiol 1994;32:277-84.
13. Miller N, Hernandez SG, Cleary TJ. Evaluation of Gen-Probe Amplified Mycobacterium Direct Test and PCR for direct detection of *Mycobacterium tuberculosis* in clinical specimens. J Clin Microbiol 1994;32:393-7.
14. Abe C, Hirano K, Wada M, Kazumi Y, Takahashi M, Fukasawa Y, Yoshimura T, Miyagi C, Goto S. Detection of *Mycobacterium tuberculosis* in clinical specimens by polymerase chain reaction and Gen-Probe Amplified *Mycobacterium Tuberculosis* Direct Test. J Clin Microbiol 1993;31:3270-4.
15. Dalovisio JR, Montenegro-James S, Kemmerly SA, Genre CF, Chambers R, Greer D, Pankey GA, Failla DM, Haydel KG, Hutchinson L, Lindley MF, Nunez BM, Praba A, Eisenach KD, Cooper ES. Comparison of the Amplified Mycobacterium Tuberculosis (MTB) Direct Test, Amplicor MTB PCR, and IS*6110*-PCR for detection of MTB in respiratory specimens. Clin Infect Dis 1996;23:1099-1106; discussion 1107-8.
16. Woods GL. Molecular techniques in mycobacterial detection. Arch Pathol Lab Med 2001;125:122-6.
17. Huang TS, Liu YC, Lin HH, Huang WK, Cheng DL. Comparison of the Roche AMPLICOR MYCOBACTERIUM assay and Digene SHARP signal system with in-house PCR and culture for detection of *Mycobacterium tuberculosis* in respiratory specimens. J Clin Microbiol 1996;34:3092-6.
18. Gamboa F, Fernandez G, Padilla E, Manterola JM, Lonca J, Cardona PJ, Matas L, Ausina V. Comparative evaluation of initial and new versions of the Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test for direct detection of *Mycobacterium tuberculosis* in respiratory and non-respiratory specimens. J Clin Microbiol 1998;36:684-9.
19. Reischl U, Lehn N, Wolf H, Naumann L. Clinical evaluation of the automated COBAS AMPLICOR MTB assay for testing respiratory and nonrespiratory specimens. J Clin Microbiol

1998;36:2853-60.
20. Thierry D, Brisson-Noel A, Vincent-Lévy-Fréebault V, Nguyen S, Guesdon JL, Gicquel B. Characterization of a *Mycobacterium tuberculosis* insertion sequence, IS*6110*, and its application in diagnosis. J Clin Microbiol 1990;28:2668-73.
21. Cave MD, Eisenach KD, McDermott PF, Bates JH, Crawford JT. IS*6110*: conservation of sequence in the *Mycobacterium tuberculosis* complex and its utilization in DNA fingerprinting. Mol Cell Probes 1991;5:73-80.
22. Brisson-Noel A, Aznar C, Chureau C, Nguyen S, Pierre C, Bartoli M, Bonete R, Pialoux G, Gicquel B, Garrigue G. Diagnosis of tuberculosis by DNA amplification in clinical practice evaluation. Lancet 1991;338:364-6.
23. Su WJ. Tsou AP. Yang MH. Huang CY. Perng RP. Clinical experience in using polymerase chain reaction for rapid diagnosis of pulmonary tuberculosis. Chin Med J 2000;63:521-6.
24. Heifets L. Dilemmas and realities of rapid diagnostic tests for tuberculosis. Chest 2000;118:4-5.
25. Peterson EM, Lu R, Floyd C, Nakasone A, Friedly G, de la Maza LM. Direct identification of *Mycobacterium tuberculosis*, *Mycobacterium avium*, and *Mycobacterium intracellulare* from amplified primary cultures in BACTEC media using DNA probes. J Clin Microbiol 1989;27:1543-7.
26. Goto M, Oka S, Okuzumi K, Kimura S, Shimada K,.Evaluation of acridinium-ester-labeled DNA probes for identification of *Mycobacterium tuberculosis* and *Mycobacterium avium-intracellulare* complex in culture. J Clin Microbiol 1991; 29:2473-6.
27. Lim SD, Todd J, Lopez J, Ford E, Janda JM. Genotypic identification of pathogenic *Mycobacterium* species by using a nonradioactive oligonucleotide probe. J Clin Microbiol 1991; 29:1276-8.
28. Small PM, van Embden JDA. Molecular epidemiology of tuberculosis. In: Bloom BR, eds. Tuberculosis: Pathogenesis, Protection and Control. Washington, DC: American Society for Microbiology Press;1994:569-82.
29. Small PM, Hoewell PC, Singh SP, Paz A, Parsonnet J, Ruston DC, Schecter GF, Daley CL, Schoolnik GK. The epidemiology of tuberculosis in San Francisco: a population-based study using conventional and molecular methods. N Engl J Med 1994;330:1703-9.
30. Daley CL, Small PM, Schecter GF, Schoolnik GK, McAdam RA, Jacobs WR Jr, Hopewell PC. An outbreak of tuberculosis with accelerated progression among persons infected with the human immunodeficiency virus: an analysis using restriction-fragment-length polymorphisms. N Engl J Med 1992;326:231-5.
31. Lemaitre N, Sougakoff W, Truffot-Pernot C, Cambaru E, Derenne JP, Bricaire F, Grosset J, Jarlier V. Use of DNA fingerprinting for primary surveillance of nosocomial tuberculosis in a large urban hospital: detection of outbreaks in homeless people and migrant workers. Int J Tuberc Lung Dis 1998;2:390-6.
32. Small PM, Shafer RW, Hopewell PC,Singh SP, Murphy MJ, Desmond E, Sierra MF, Schoolnik GK. Exogenous reinfection with multidrug-resistant *Mycobacterium tuberculosis* in patients with advanced HIV infection. N Engl J Med 1993;328:1137-44.
33. Bauer J, Thomsen V, Poulsen S, Andersen AB. False-positive results from cultures of *Mycobacterium tuberculosis* due to laboratory cross-contamination confirmed by restriction fragment length polymorphism. J Clin Microbiol 1997;35:988-991.
34. Cohn DL, O'Brien RJ. The use of restriction fragment length polymorphism (RFLP) analysis for epidemiological studies of tuberculosis in developing countries. Int J Tuberc Lung Dis 1998;2:16-26.
35. van Embden JDA, Cave MD, Crawford JT, Dale JW, et al. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. J Clin Microbiol 1993;31:406-9.
36. van Soolingen D, Hermans PWM, de Haas PEW, Soll DR, van Embden JD. Occurrence and stability of insertion sequences in *Mycobacterium tuberculosis* complex strains: evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. J Clin Microbiol 1991;29:2578-86.
37. Vauterin P. Sharing fingerprint databases among different laboraties: problems and solutions. Proceedings of Second Meeting of the European Community Concerted Action on Genetic Markers and Tuberculosis Epidemiology, Institut Pasteur, Paris, November 1995.
38. Hermans PWM, van Soolingen D, Bik EM, de Haas PEW, Dale JW, van Embden JDA. The insertion element, IS987, from *Mycobacterium bovis* BCG is located in a hot spot integration region for insertion elements in *Mycobacterium tuberculosis* complex strains. Infect Immun 1991;59:2695-2705.
39. Kamerbeek J, Schouls L, Kolk A, van Agterveld M, van Soolingen D, Kuijper S, Bunschoten A, Molhuizen H, Shaw R, Goyal M, van Embden J. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J Clin Microbiol 1997;35:907-14.
40. Ross BC, Raios K, Jackson K, Dwyer B. Molecular cloning of a highly repeated DNA element from *Mycobacterium tuberculosis* and its use as an epidemiological tool. J Clin Microbiol 1992;30:942-6.
41. Chaves F, Yang Z, Hajj HE, Alonso M, Burman WJ, Eisenach KD, Dronda F, Bates JH, Cave MD. Usefulness of the secondary probe pTBN12 in DNA fingerprinting of *Mycobacterium tuberculosis*. J Clin Microbiol 1996;34:1118-23.
42. Victor TC, Warren R, Butt JL, Jordaan AM, Felix JV, Venter A, Sirgel FA, Schaaf HS, Donald PR, Richardson M, Cynamon MH, Van Helden PD. Genome and MIC stability in *Mycobacterium tuberculosis* and indications for continuation of use of isoniazid in multidrug-resistant tuberculosis. J Med Microbiol 1997;46:847-957.
43. Rattan A, Kalia A, Ahmad N. Multidrug-resistant *Mycobacterium tuberculosis*: molecular perspectives. Emerg Infect Dis 1998;4:195-209.
44. Musser JM. Antimicrobial agent resistance in mycobacteria: molecular genetic insights. Clin Microbiol Rev 1995;8:496-514.
45. Telenti A, Imboden P, Marchesi F, Lowrie D, Cole S, Colston MJ, Matter L, Schopfer K, Bodmer T. Detection of rifampicin resistant mutations in *Mycobacterium tuberculosis*. Lancet 1993;341:647-50.
46. Williams DL, Waguespack C, Eisenach K, *et al.* Characterization of rifampin resistance in pathogenic mycobacteria. Antimicrob Agents Chemother 1994;38:2380-6.
47. Telenti A, Imboden P, Marchesi F, Schmidheini T, Bodmer T. Direct automated detection of rifampin-resistant *Mycobacterium tuberculosis* by polymerase chain reaction and single strand conformation polymorphism analysis. Antimicrob Agents

Chemother 1993;37:2054-8.
48. De Beenhouwer H, Lhiang Z, Jannes G, Mijs W, Machtelinckx L, Rossau R, Traore H, Portaels F, Mijs W, Machtelinckx L, Rossau R, Traore H, Portaels F. Rapid detection of rifampicin resistance in sputum and biopsy specimens from tuberculosis patients by PCR and line probe assay. Tuber Lung Dis 1995;76:425-30.
49. Watterson SA, Wilson SM, Yates MD, Drobniewski FA. Comparison of three molecular assays for rapid detection of rifampicin resistance in *Mycobacterium tuberculosis*. J Clin Microbiol 1998;36:1969-73.
50. Victor TC, Jordaan AM, van Rie A, van der Spuy GD, Richardson M, van Helden PD, Warren R. Detection of mutations in drug tesistance genes of *Mycobacterium tuberculosis* by a dot-blot hybridization strategy. Tuber Lung Dis 1999;79:343-8.
51. Ramaswamy S, Musser JM. Molecular genetic basis of antimicrobial agent resistance in *Mycobacterium tuberculosis*:1998 update. Tuber Lung Dis 1998;79:3-29.
52. Telenti A, Persing DH. Novel strategies for the detection of drug resistance in *Mycobacterium tuberculosis*. Res Microbiol 1996;147:73-9.
53. Mikhailovich V, Lapa S, Gryadunov D, Sobolev A, Strizhkov B, Chernyh N, Skotnikova O, Irtuganova O, Moroz A, Litvinov V, Vladimirskii M, Perelman M, Chernousova L, Erokhin V, Zasedatelev A, Mirzabekov A. Identification of rifampin-resistant *Mycobacterium tuberculosis* strains by hybridization, PCR, and ligase detection reaction on oligonucleotide microchips. J Clin Microbiol 2001;39:2531-40.
54. Gingeras TR, Ghandour G, Wang E, Berno A, Small PM, Drobniewski F, Alland D, Desmond E, Holodniy M, Drenkow J. Simultaneous genotyping and species identification using hybridization pattern recognition analysis of generic *Mycobacterium* DNA arrays. Genome Res 1998;8:435-48.
55. Troesch A, Nguyen H, Miyada CG, Desvarenne S, Gingeras TR, Kaplan PM, Cros P, Mabilat C. *Mycobacterium* species identification and rifampin resistance testing with high-density DNA probe arrays. J Clin Microbiol 1999;37:49-55.