Comparison of an Automated Repetitive Sequence-based PCR Microbial Typing System with IS6110-Restriction Fragment Length Polymorphism for Epidemiologic Investigation of Clinical Mycobacterium tuberculosis Isolates in Korea

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Tuberculosis remains a severe public health problem worldwide. Presently, genotyping is used for conducting epidemiologic and clinical studies on tuberculosis cases. We evaluated the efficacy of the repetitive sequence-based PCR (rep-PCR)-based DiversiLab™ system (bioMérieux, France) over the IS6110-restriction fragment length polymorphism analysis for detecting Mycobacterium tuberculosis. In all, 89 clinical M. tuberculosis isolates collected nationwide from Korea were used. The DiversiLab system allocated the 89 isolates to 8 groups with 1 unique isolate when a similarity level of 95% was applied. Seventy-six isolates of the Beijing family and 13 isolates of non-Beijing family strains were irregularly distributed regardless of rep-PCR groups. The DiversiLab system generated a rapid, sensitive, and standardized result. It can be used to conduct molecular epidemiologic studies to identify clinical M. tuberculosis isolates in Korea.

Key Words: Mycobacterium tuberculosis, Repetitive sequence-based polymerase chain reaction, DiversiLab Microbial Typing System

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paring it with previous data from IS6110-RFLP analysis [9]. We studied 89 clinical *M. tuberculosis* isolates collected from 11 university hospitals nationwide in Korea between 2008 and 2009 from epidemiologically unrelated patients. All 89 isolates had already been characterized by IS6110-RFLP [9].

According to the manufacturer's recommendations, the DiversiLab Fingerprinting Kit (Bacterial Barcodes, Inc., Houston, TX, USA) was used for rep-PCR amplification of non-coding intergenic repetitive elements in the genomic DNA of the isolates. Rep-PCR genomic typing was performed using the DiversiLab system. The DiversiLab system includes fragment separation using microfluidic chips (LabChip device; Caliper Technologies, Inc., Mountain View, CA, USA) and the Agilent B2100 Bioanalyzer (Agilent Technologies Inc., Palo Alto, CA, USA). Results were analyzed using DiversiLab software (version 3.3). Reports, including dendrograms, electropherograms, and virtual gel and scatterplot images, were generated automatically. To evaluate reproducibility, we performed the entire assay, including DNA extraction, PCR, and chip analysis on the bioanalyzer, in duplicate. The percent similarities of the dendrograms were analyzed by Pearson correlation. We used a single numerical index of discrimination on the basis of Simpson's index of diversity [10].

The results of the DiversiLab system defined 89 isolates in 8 groups and 1 unique isolate when a similarity level of 95% was applied (Fig. 1). One major group contained 18 isolates. Seventy-six isolates of the Beijing family and 13 isolates of non-Beijing family strains, previously defined by IS6110-RFLP [1], were distributed to each group. Therefore, this method was able to discriminate strain variations between Beijing family members. Among the 14 isolates of the K family, which showed unique RFLP patterns and are rather highly prevalent in Korea, 7 (50%) belonged to 1 major group. The reproducibility studies showed identical rep-PCR patterns for each duplicate test with the same strain. The results of cluster analysis did not correlate with any region of strain isolation.

Few publications are available on the performance of the DiversiLab system [11-13]. Cangelosi et al. [14] evaluated the system as an epidemiologic method for *M. tuberculosis* and *M. avium* complex, indicating that it can replace IS6110-RFLP for typing *M. tuberculosis*. In our current study, we showed the usefulness of the DiversiLab system by evaluating its efficacy with 89 *M. tuberculosis* isolates collected nationwide in Korea. The system displayed highly discriminatory results among Beijing family strains that have shown nearly identical IS6110-RFLP patterns. IS6110-RFLP is considered a gold standard method in strain typing for *M. tu-

![Fig. 1. Comparison of rep-PCR results with IS6110-RFLP patterns for 89 clinical Mycobacterium tuberculosis isolates. A scale for rep-PCR similarity (%) is shown at the bottom of the figure. IS6110-RFLP results are cited from reference 9. Abbreviations: RFLP, restriction fragment length polymorphism; NB, strains of non-Beijing family; B, strains of Beijing family; K, strains of K family.](image)

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VNTR) is fairly rapid, requires only small amounts of DNA, and can easily be digitized to share data among laboratories [17]. Single-nucleotide polymorphism (SNP) analysis also provides a powerful strategy for large-scale molecular population studies examining phylogenetic relations among bacterial strains [18, 19]. However, the DiversiLab system provides results within 24 hr. Moreover, the DiversiLab system can generate a sensitive and standardized result and is reproducible and highly discriminatory. Thus, it can be used in the molecular epidemiologic investigation of clinical M. tuberculosis isolates in Korea.

Authors’ Disclosures of Potential Conflicts of Interest
No potential conflict of interest relevant to this article was reported.

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REFERENCES
1. Global tuberculosis control: key findings from the December 2009 WHO report. Wkly Epidemiol Rec 2010;85:69-80.
2. Lönnroth K and Raviglione M. Global epidemiology of tuberculosis: prospects for control. Semin Respir Crit Care Med 2008;29:481-91.
3. Espinal MA, Laszlo A, Simonsen L, Boulahbal F, Kim SJ, Reniero A, et al. Global trends in resistance to antituberculosis drugs. World Health Organization-International Union against Tuberculosis and Lung Disease Working Group on Anti-Tuberculosis Drug Resistance Surveillance. N Engl J Med 2001;344:1294-303.
4. Gopaul KK, Brown TJ, Gibson AL, Yates MD, Drobniewski FA. Progression toward an improved DNA amplification-based typing technique in the study of Mycobacterium tuberculosis epidemiology. J Clin Microbiol 2006;44:2492-8.
5. Dombek PE, Johnson LK, Zimmerley ST, Sadowsky MJ. Use of repetitive DNA sequences and the PCR to differentiate Escherichia coli isolates from human and animal sources. Appl Environ Microbiol 2000;66:2572-7.
6. Healy M, Huang J, Bittner T, Lising M, Frye S, Raza S, et al. Microbial DNA typing by automated repetitive-sequence-based PCR. J Clin Microbiol 2005;43:199-207.
7. Carretto E, Barbarini D, Farina C, Grosini A, Nicoletti P, Manso E. Use of the DiversiLab semiautomated repetitive-sequence-based polymerase chain reaction for epidemiologic analysis on Acinetobacter baumannii isolates in different Italian hospitals. Diagn Microbiol Infect Dis 2008;60:1-7.
8. van Embden JD, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B, et al. Strain identification of Mycobacterium tuberculosis by DNA fingerprinting: recommendations for a standardized methodology. J Clin Microbiol 1993;31:406-9.
9. Choi GE, Jang MH, Song EJ, Jeong SH, Kim JS, Lee WG, et al. IS6110-restriction fragment length polymorphism and spoligotyping analysis of Mycobacterium tuberculosis clinical isolates for investigating epidemiologic distribution in Korea. J Korean Med Sci 2010;25:1716-21.
10. Hunter PR and Gaston MA. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J Clin Microbiol 1988;26:2465-6.
11. Versalovic J, Koeuth T, Lupski JR. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Res 1991;19:6823-31.
12. Versalovic J, Kapur V, Mason EO Jr, Shah U, Koeuth T, Lupski JR, et al. Penicillin-resistant Streptococcus pneumoniae strains recovered in Houston: identification and molecular characterization of multiple clones. J Infect Dis 1993;167:850-6.
13. Versalovic J, Kapur V, Koeuth T, Mazurek GH, Whittam TS, Musser JM, et al. DNA fingerprinting of pathogenic bacteria by fluorophore-enhanced repetitive sequence-based polymerase chain reaction. Arch Pathol Lab Med 1995;119:23-9.
14. Cangelosi GA, Freeman RJ, Lewis KN, Livingston-Rosanoff D, Shah KS, Milan SJ, et al. Evaluation of a high-throughput repetitive-sequence-based PCR system for DNA fingerprinting of Mycobacterium tuberculosis and Mycobacterium avium complex strains. J Clin Microbiol 2004;42:2685-93.
15. van Soolingen D, Qian L, de Haas PE, Douglas JT, Traore H, Portaels F, et al. Predominance of a single genotype of Mycobacterium tuberculosis in countries of East Asia. J Clin Microbiol 1995;33:3234-8.
16. Shamputa IC, Lee J, Allix-Béguec C, Cho EJ, Lee HI, Rajan V, et al. Genetic diversity of Mycobacterium tuberculosis isolates from a tertiary care tuberculosis hospital in South Korea. J Clin Microbiol 2010;48:387-94.
17. Supply P, Allix C, Lesjean S, Cardoso-Oelmann M, Rüscher-Gerdes S, Willery E, et al. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of Mycobacterium tuberculosis. J Clin Microbiol 2006;44:4498-510.
18. Bouakaze C, Keyser C, de Martino SJ, Sougakoff W, Veiziris N, Dabernat H, et al. Identification and genotyping of Mycobacterium tuberculosis complex species by use of a SNAPSHOT Minisequencing-based assay. J Clin Microbiol 2010;48:1758-66.
19. Choi GE, Jang MH, Cho HJ, Lee SM, Yi J, Lee EY, et al. Application of single-nucleotide polymorphism and mycobacterial interspersed repetitive units-variable number of tandem repeats analyses to clinical Mycobacterium tuberculosis isolates from Korea. Korean J Lab Med 2011;31:37-43.