

## Short Communication

### FTA Card Utility for PCR Detection of *Mycobacterium leprae*

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**SUMMARY:** The suitability of the FTA® elute card for the collection of slit skin smear (SSS) samples for PCR detection of *Mycobacterium leprae* was evaluated. A total of 192 SSS leprosy samples, of bacillary index (BI) 1 to 5, were collected from patients attending two skin clinics in Myanmar and preserved using both FTA® elute cards and 70% ethanol tubes. To compare the efficacy of PCR detection of DNA from each BI class, PCR was performed to amplify an *M. leprae*-specific repetitive element. Of the 192 samples, 116 FTA® elute card and 112 70% ethanol samples were PCR positive for *M. leprae* DNA. When correlated with BI, area under the curve (AUC) values of the respective receiver-operating characteristic curves were similar for the FTA® elute card and ethanol collection methods (AUC = 0.6). Taken together, our results indicate that the FTA® elute card, which enables the collection, transport, and archiving of clinical samples, is an attractive alternative to ethanol preservation for the detection of *M. leprae* DNA.

In January 2010, the global registered prevalence of leprosy was 211,903 cases (1). To meet future challenges and to sustain the trend of decline in leprosy cases, WHO has developed simple guidelines for annual routine sentinel surveillance programs to monitor drug resistance (2). These programs detect *Mycobacterium leprae* drug resistance mutations using direct PCR sequencing of the drug resistance-determining regions (DRDR) of relevant genes. While most *M. leprae* samples are currently stored in 70% ethanol until laboratory analysis, the FTA® elute card (Cat. no. WB120401; Whatman Inc., Florham Park, N.J., USA) represents an alternative method for the collection and safe transportation of leprosy samples (3).

FTA® elute cards are designed for room temperature collection, shipment, archiving, and purification of nucleic acids from biological samples for PCR analysis. However, no studies analyzing the suitability of the FTA® elute card for preservation of leprosy slit skin smear (SSS) samples to detect *M. leprae* DNA have been reported thus far.

In this study, to compare the efficacy of PCR using DNA samples recovered from FTA® elute cards and from 70% ethanol, we performed nested PCR to amplify the *M. leprae*-specific repetitive element (RLEP). This is a highly sensitive method routinely used in molecular epidemiology for the detection of *M. leprae* DNA. In addition, the suitability of the FTA® elute

card for the collection of SSS samples for PCR detection of *M. leprae* was evaluated.

In 2009, 192 multibacillary leprosy patients, with bacterial indices (BI) of 1 to 5, were recruited from the Central Special Skin Center at Yangon General Hospital and from Mandalay General Hospital. After giving informed consent, each patient submitted two SSS samples, which were preserved on an FTA® elute card or in 70% ethanol using separate sterile disposable blades. The first SSS sample was smeared directly onto an FTA® elute card. The second SSS was scraped from the same site and the blade was then immersed in a tube containing 1 ml of 70% ethanol. Samples were stored at room temperature until the tests were performed at the Department of Medical Research, Lower Myanmar (DMR). Forty-four randomly selected DNA samples were sent to the Leprosy Research Center (LRC) at the National Institute of Infectious Diseases (NIID), Tokyo, Japan, for external quality control. The research proposal was approved by the Institutional Ethical Review Committee at DMR. PCR efficacy of the samples harvested using the FTA® elute card or using ethanol was compared for each BI category.

DNA from SSS specimens preserved in 70% ethanol was extracted according to Klatser's method (4). Briefly, sample tubes were centrifuged at 18,000 × g for 10 min, the supernatant was discarded, and the precipitate was suspended in phosphate buffered saline (PBS) (pH 7.2) and allowed to stand for 30 min. The suspension was then centrifuged at 18,000 × g for 10 min to remove remaining alcohol. The washed precipitate was suspended in 50 µl of lysis buffer containing 10 mg/ml proteinase K in 1 M Tris-HCL, pH 8.5, and 0.5% Tween 20, and incubated at 60°C for 18 h. After boiling at 97°C for 10 min, samples were frozen and thawed

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twice to disrupt bacilli completely. DNA was retrieved from FTA® elute cards according to the manufacturer's directions. Briefly, each was dried in a 60°C incubator for 30 min. The sample area was then cut out using a disposable 4-mm diameter biopsy punch, washed with 500 µl of sterile water, and then suspended in 30 µl of sterile water and heated at 95°C for 25 min. The eluted DNA was stored at -20°C until PCR analysis.

Nested PCR assay was performed to amplify RLEP. The RLEP element was targeted because its copy number in the *M. leprae* genome is known to be more than 35 (5,6). In the first round of PCR, a total reaction volume of 25 µl was used, containing 1 µl of SSS DNA as the template. The second round of PCR used the same reaction volume, and contained 1 µl of the outer PCR product, diluted 1:50 in sterile water, as the template (7). PCR was performed using the following conditions: initial 94°C denaturation for 4 min; 25 cycles of denaturation (95°C for 40 s), annealing (55°C for 1 min), and elongation (72°C for 20 s); and final elongation (72°C for 1 min). Distilled water and DNA from the *M. leprae* Thai-53 strain (8), purified at NIID, served as negative and positive controls, respectively. The PCR products were visualized by gel electrophoresis.

The data were analyzed using a statistical software package (version 9.3.2.0; MedCalc® software [http://www.medcalc.be]) and a receiver operating characteristic (ROC) curve was drawn (9). Additionally, a statistically significant difference between assays was confirmed by the chi-square test (10).

The number of samples in each BI category is shown in Table 1. Of the 192 samples, 116 samples from the FTA® elute cards (60%) and 112 samples from 70% ethanol (58%) were nested PCR positive for RLEP. When correlated with BI classification, 35% of the FTA® elute card and 45% of the 70% ethanol BI-1 samples were positive for *M. leprae*, 56 and 46% in BI-2, 57% each in BI-3, 59 and 62% in BI-4, and 82 and 77% in BI-5 or more, respectively (Table 1). The area under the curve (AUC) values of FTA® elute card and ethanol tubes ROC curves were similar (AUC = 0.6), and each method showed a statistically significant positive correlation between BI value and detection of *M. leprae* DNA by PCR ( $P = 0.01$ ).

To test the reproducibility of FTA® elute card PCR efficacy, 22 DNA samples were randomly selected from each of the FTA® elute card- and 70% ethanol-harvested groups (approximately 10% of total samples) and sent to the LRC at the NIID for analysis. Twenty of the 22 samples in each group were PCR positive for *M. leprae*.

Table 1. Positive rate of PCR from FTA® elute card sample and 70% ethanol sample

Bacillary index ( $n$ ) <sup>1)</sup>	FTA elute card (%)	70% ethanol (%)
BI-1 (31)	11 (35)	14 (45)
BI-2 (48)	27 (56)	22 (46)
BI-3 (29)	17 (57)	17 (57)
BI-4 (37)	22 (59)	23 (62)
BI-5 & >(47)	39 (82)	36 (77)
Total (192)	116 (60)	112 (58)

<sup>1)</sup>: Samples for each BI class.

Molecular detection from various samples collected using the FTA® elute card has been reported previously (11–13). In this study, the FTA® elute card was evaluated as a collection medium for SSS leprosy samples, and 60% of the FTA® elute card-harvested samples were found to be PCR positive for *M. leprae* DNA, as compared to 58% of ethanol-harvested samples. Moreover, the AUC values of the ROC curves were similar between FTA® elute card and 70% ethanol samples (AUC = 0.6), when correlated with BI. Taken together, our results indicate that the FTA® elute card provides a fast and reliable method for sample collection and DNA extraction for the detection of *M. leprae* that can replace 70% ethanol collection methods.

Using FTA® elute cards, DNA remains detectable by PCR more than 4 years after specimen collection (14), and the manufacturer claims to have obtained PCR amplifiable DNA after 14 years of storage. DNA elution from FTA® elute cards has several advantages over purification from 70% ethanol, involving a simple water/heat protocol that does not require special reagents or equipment. Direct sequencing of PCR products is widely used for *M. leprae* genotyping and drug resistance identification (15,16). The FTA® elute card provides both a small format and room temperature stability, making it particularly suitable for the collection of samples for *M. leprae* screening.

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**Conflict of interest** None to declare.

## REFERENCES

- World Health Organization (2010): Global leprosy situation, 2010. Wkly. Epidemiol. Rec., 85, 337–348.
- World Health Organization Regional Office for South-East Asia (2009): Guidelines for Global Surveillance of Drug Resistance in Leprosy. SEA-GLP., 2009.2
- Matsuoka, M., Khin, S.A., Kyaw, K., et al. (2008): A novel method for simple detection of mutations conferring drug resistance in *Mycobacterium leprae*, based on a DNA microarray, and its applicability in developing countries. J. Med. Microbiol., 57, 1213–1219.
- De Wit, M.Y.L., Faber, W.R., Krieg, S.R., et al. (1991): Application of a polymerase chain reaction for the detection of *Mycobacterium leprae* in skin tissue. J. Clin. Microbiol., 29, 900–905.
- Cole, S.T., Supply, P. and Honore, N. (2001): Repetitive sequences in *Mycobacterium leprae* and their impact on genome plastic. Lepr. Rev., 72, 449–461.
- Kai, M., Nguyen Phuc, N.H., Nguyen Hoang, A., et al. (2011): Analysis of drug-resistant strains of *Mycobacterium leprae* in an endemic area of Vietnam. Clin. Infect. Dis., 52, e127–e132.
- Donoghue, H.D., Holton, J. and Spigelman, M. (2001): PCR primers that can detect low levels of *Mycobacterium leprae* DNA. J. Med. Microbiol., 50, 177–182.
- Matsuoka, M. (2010): The history of *Mycobacterium leprae* Thai-53 strain. Lep. Rev., 81, 137.
- Beck, J.R. and Schultz, E.K. (1986): The use of relative operating characteristic (ROC) curves in test performance evaluation. Arch. Pathol. Lab. Med., 110, 13–20.
- Siegel, S. and Castellan, N. J., Jr. (1988): Non-Parametric Statistics for the Behavioral Sciences. 2nd ed. McGraw-Hill, New York, N.Y.
- Lema, C., Kohl-White, K., Lewis, L.R., et al. (2006): Optimized pH method for DNA elution from buccal cells collected in Whatman FTA cards. Genet. Test, 10, 126–130.

12. Milne, E., van Bockxmeer, F.M., Robertson, L., et al. (2006): Buccal DNA collection: comparison of buccal swabs with FTA cards. *Cancer Epidemiol. Biomarkers Prev.*, 15, 816–819.
13. Gustavsson, I., Lindell, M., Wilander, E., et al. (2009): Use of FTA card for dry collection, transportation and storage of cervical cell specimen to detect high-risk HPV. *J. Clin. Virol.*, 46, 112–116.
14. Smith, L.M. and Burgoyne, L.A. (2004): Collecting, archiving and processing DNA from wild life samples using FTA data basing paper. *BMC Ecol.*, 4, 4.
15. Matsuoka, M. (2010): Drug resistance in Leprosy. *Jpn. J. Infect. Dis.*, 63, 1–7.
16. Gillis, T., Vissa, V., Matsuoka, M., et al. (2009): Characterisation of short tandem repeats for genotyping *Mycobacterium leprae*. *Lepr. Rev.*, 80, 250–260.