149 **DISCUSSION**

150 To functionally replace the *rpoB* gene of *M. smegmatis* with the *M. leprae* or *M*
151 *tuberculosis* counterpart, we used the method established in our previous study (16).
152 Because *rpoB* is a necessary gene for bacterial growth, this genetic locus cannot be
153 disrupted without compensating for its activity. Therefore, we first introduced the *rpoB*
154 gene of *M. leprae* or *M. tuberculosis* into *M. smegmatis* using vector plasmids of two
155 types before disrupting the *rpoB* gene on the *M. smegmatis* chromosome. One vector
156 was pMV261, a multi-copy shuttle plasmid. The other was a single-copy integrative
157 shuttle plasmid pNN301. However, isolation of *rpoB*-disrupted mutants carrying
158 pNN301-*rpoB* constructs was unsuccessful, probably because of insufficient RpoB
159 expression.

160 We tested 2 silent mutations and 10 mutations that change amino-acid residues for
161 *M. leprae*. Codons 516, 526, 531, and 533 in the *M. leprae rpoB* are known as
162 responsible codons for rifampicin resistance. However, it remains unclear whether or
163 not mutations that have not been reported previously can confer rifampicin resistance.
164 Our results show that not all mutations in the *rpoB* gene detected in *M. leprae* clinical
165 samples confer rifampicin resistance. *M. leprae* is not cultivable. Therefore, it has been
166 very difficult to analyze the mutation-susceptibility relation. Using recombinant *M.*
167 *smegmatis*, however, we can analyze it in a few weeks. We also tested 1 silent mutation
168 and 24 mutations that change amino acids, 2 deletions, and 1 insertion for *M.*
169 *tuberculosis*. Some mutations did not confer rifampicin resistance, which is inconsistent
170 with susceptibility of the *M. tuberculosis* clinical isolates reported previously. Most
171 mutations at codon 516, 526, or 531 showed rifampicin resistance. It is interesting that
172 the strains with mutation GAC516→CAC for D516H were not rifampicin resistant. All

173 other mutations at codon 516 showed rifampicin resistance. Mutation GAC516→CAC
174 in *M. tuberculosis* was reported in a strain with multiple mutations and should not be
175 involved in rifampicin resistance.

176 Rifabutin, a spiro-piperidyl rifampicin, is a rifamycin derivative, which is more
177 active than rifampicin against slow-growing mycobacteria, including *M. tuberculosis*
178 and *M. avium-intracellulare* complex, *in vitro* and *in vivo*. It is also active against some
179 rifampicin-resistant strains of *M. tuberculosis* (6, 13). Our results indicate that some
180 mutations (e.g. GAT516→AAT of *M. leprae* and GAC516→GAG of *M. tuberculosis*)
181 show weak resistance to rifabutin.

182 Molecular methods designed to detect drug resistance have some limitations. In
183 some cases, identified mutations are not related to the acquisition of resistance. Caution
184 is necessary when considering mutations, especially if the mutation detected in clinical
185 isolates is not reported very often. For example, mutations for Q510H and L521M were
186 detected in rifampicin-resistant *M. tuberculosis* isolates (21, 22), but our results suggest
187 that these mutations are not responsible for rifampicin resistance (Table 2). The method
188 used for this study can directly assess the influence of designated mutations in *rpoB*. If
189 the mutations can confer rifampicin resistance, then we can eliminate the possibility that
190 genetic variation in some other regions than *rpoB* on the chromosome of the clinical
191 isolates is responsible for the resistance. Bahrmand *et al.* reported high-level rifampicin
192 resistance of *M. tuberculosis* isolates with multiple mutations within the *rpoB* gene (1).
193 Our method might also be useful for analyzing multiple mutations detected in the *rpoB*
194 gene of clinical isolates to determine the contribution of each single mutation to
195 rifampicin resistance.
196

197 **ACKNOWLEDGMENTS**

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200

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279 **FIGURE LEGENDS**

280

281 Figure 1. Construction of recombinant *M. smegmatis* strains for rifampicin
282 susceptibility testing.

283

284 Figure 2. Mutations introduced into the *M. leprae rpoB* gene or *M. tuberculosis rpoB*
285 gene and rifampicin susceptibility. The consensus amino acid sequence of the *M. leprae*
286 RpoB and *M. tuberculosis* RpoB between codons 506 and 565 is shown. The *M. leprae*
287 *rpoB* sequence and codons are shown above the consensus amino acid sequence. The *M.*
288 *tuberculosis rpoB* sequence and codons are shown below the consensus sequence.
289 Mutated codons that gave rise to rifampicin resistance are surrounded by ovals. Mutated
290 codons that showed comparable levels of rifampicin susceptibility to those of the wild
291 type sequences are surrounded by rectangles.

Table 1 Primers used for this study

Primer	Sequence[a]	Application
for *M. smegmatis*		
MSRBUF	GCCTTAAGGAGGAGAAGGACGAGGCCAC	*rpoB* disruption, upstream forward
MSRBUR	GCTCTAGACAAGATGCATCCTTCCAGCA	*rpoB* disruption, upstream reverse
MSRBDF	GCAAGCTTTCGCGCAACGAATCCGCGTC	*rpoB* disruption, downstream forward
MSRBDR	GCACTAGTAGCGCACGCAGCTTCTTCTG	*rpoB* disruption, downstream reverse
MSRBF	TGGTCAAGCAGTTCCTCAAC	detection of *rpoB* disruption, forward
MSRBR	CGTTGTTGACGATGATCTCG	detection of *rpoB* disruption, reverse
for *M. leprae*		
MLRBWTF	GCGGATCCGTGCTGGAAGGATGCATCTT	cloning of *M. leprae rpoB*, forward
MLRBWTR	GCGTTAACCTAAGCCAGATCTTCTATGG	cloning of *M. leprae rpoB*, reverse
MLRBWTF1	CAGTTCATGGATCAGAACAACCCTC	introduction of point mutation at codons 507 and 508
MLRBWTF2	TGTCGGCGCTGGGCCCGGGTGGTTT	introduction of point mutation at codon 526
MLRBWTF3	TTCGCACTACGGCCGGATGTGCCCG	introduction of point mutation at cidib 547
MLRBWTR1	CGACAGCTGGCTGGTGCCGAAGAAT	introduction of point mutation at codons 513, 516, and 517
MLRBWTR2	GCCGGCGCTTGTGGGTCAGGCCCGA	introduction of point mutation at codons 531, 532, and 533
MLRB507GGG	CGACAGCTGGCTGGTCCCGAAGAAT	introduction of point mutation GGC507→GGG
MLRB507AGC	CGACAGCTGGCTGGTGCTGAAGAAT	introduction of point mutation GGC507→AGC
MLRB508ACA	CGACAGCTGGCTTGTGCCGAAGAAT	introduction of point mutation ACC508→ACA
MLRB513GTG	GTGTTCATGGATCAGAACAACCCTC	introduction of point mutation CAG513→GTG
MLRB516AAT	CAGTTCATGAATCAGAACAACCCTC	introduction of point mutation GAT516→AAT
MLRB517CAT	CAGTTCATGGATCATAACAACCCTC	introduction of point mutation CAG517→CAT
MLRB526TAC	GCCGGCGCTTGTAGGTCAGGCCCGA	introduction of point mutation CAC526→TAC
MLRB531TTG	TGTTGGCGCTGGGCCCGGGTGGTTT	introduction of point mutation TCG531→TTG
MLRB531TGG	TGTGGGCGCTGGGCCCGGGTGGTTT	introduction of point mutation TCG531→TGG
MLRB532TCG	TGTCGTCGCTGGGCCCGGGTGGTTT	introduction of point mutation GCG532→TCG
MLRB533CCG	TGTCGGCGCCGGGCCCGGGTGGTTT	introduction of point mutation CTG533→CCG
MLRB547ATC	GGGTGCACGTCACGGATCTCTAGCC	introduction of point mutation GTC547→ATC
for *M. tuberculosis*		
MTRBWTF	GCGAATTCTTGGCAGATTCCCGCCAGAG	cloning of *M. tuberculosis rpoB*, forward
MTRBWTR	GCAAGCTTTTACGCAAGATCCTCGACAC	cloning of *M. tuberculosis rpoB*, reverse

[a] Restriction sites are underlined

Table 1 (continued) Primers used for this study

Primer	Sequence	Application
MTRBWTF1	AATTCATGGACCAGAACAACCCGCT	introduction of point mutation at codons 507, 508, 510, 511, 512, and 513 deletion of codons 506–508
MTRBWTF2	CTGTCGGCGCTGGGGCCCGGCGGTC	introduction of point mutation at codons 522, 523, 526, and 531
MTRBWTR1	GGCTCAGCTGGCTGGTGCCGAAGAA	introduction of mutation at codons 514, 516, 518, 519, and 521 deletion of codon 518, and insertion of TTC between codons 514 and 515
MTRBWTR2	TCGGCGCTTGTGGGTCAACCCCGAC	introduction of point mutation TCG531→TTC and TCG531→TTG
MTRB507AGC	GGCTCAGCTGGCTGGTGCTGAAGAA	introduction of point mutation GGC507→AGC
MTRB507GAT	GGCTCAGCTGGCTGGTATCGAAGAA	introduction of point mutation GGC507→GAT
MTRB508CAC	GGCTCAGCTGGCTGTGGCCGAAGAA	introduction of point mutation ACC508→CAC
MTRB508GCC	GGCTCAGCTGGCTGGCGCCGAAGAA	introduction of point mutation ACC508→GCC
MTRB510CAT	GGCTCAGATGGCTGGTGCCGAAGAA	introduction of point mutation CAG510→CAT
MTRB511CCG	GGCTCGGCTGGCTGGTGCCGAAGAA	introduction of point mutation CTG511→CCG
MTRB513AAT1	TGCTCAGCTGGCTGGTGCCGAAGAA	introduction of point mutation CAA513→AAT
MTRB513AAT2	ATTTCATGGACCAGAACAACCCGCT	introduction of point mutation CAA513→AAT
MTRB513GAA	CGCTCAGCTGGCTGGTGCCGAAGAA	introduction of point mutation CAA513→GAA
MTRB516GAG	AATTCATGGAGCAGAACAACCCGCT	introduction of point mutation GAC516→GAG
MTRB516CAC	AATTCATGCACCAGAACAACCCGCT	introduction of point mutation GAC516→CAC
MTRB516GTC	AATTCATGGTCCAGAACAACCCGCT	introduction of point mutation GAC516→GTC
MTRB521ATG	AATTCATGGACCAGAACAACCCGAT	introduction of point mutation CTG521→ATG
MTRB522TTG	TCGGCGCTTGTGGGTCAACCCCAAC	introduction of point mutation TCG522→TTG
MTRB523GCG	TCGGCGCTTGTGGGTCAACGCCGAC	introduction of point mutation GGG523→GCG
MTRB523GGC	TCGGCGCTTGTGGGTCAAGCCCGAC	introduction of point mutation GGG523→GGC
MTRB526CTC	TCGGCGCTTGAGGGTCAACCCCGAC	introduction of point mutation CAC526→CTC
MTRB526TAC	TCGGCGCTTGTAGGTCAACCCCGAC	introduction of point mutation CAC526→TAC
MTRB526GAC	TCGGCGCTTGTCGGTCAACCCCGAC	introduction of point mutation CAC526→GAC
MTRB526TTC	TCGGCGCTTGAAGGTCAACCCCGAC	introduction of point mutation CAC526→TTC
MTRB526AAC	TCGGCGCTTGTTGGTCAACCCCGAC	introduction of point mutation CAC526→AAC
MTRB526CGC	TCGGCGCTTGCGGGTCAACCCCGAC	introduction of point mutation CAC526→CGC
MTRB526CAA	TCGGCGCTTTTGGGTCAACCCCGAC	introduction of point mutation CAC526→CAA
MTRB529AAA	TTTGCGCTTGTGGGTCAACC	introduction of point mutation CGA529→AAA
MTRB531TTC	CTGTTCGCGCTGGGGCCCGGCGGTC	introduction of point mutation TCG531→TTC

Table 1 (continued) Primers used for this study

Primer	Sequence	Application
MTRB531TTG	CTGTTGGCGCTGGGGCCCGGCGGTC	introduction of point mutation TCG531→TTG
MTRB506d	GGCTCAGCTGGCTGAACTCCTTGAT	introduction of mutation 506-508del
MTRBin514TTC	AATTCTTCATGGACCAGAACAACCC	introduction of mutation 514insTTC
MTRBd518	AATTCATGGACCAGAACCCGCTGTC	introduction of mutation 518del

366 Table 2 Rifampicin and Rifabutin susceptibilities of the recombinant *M. smegmatis*
367 strains
368

Mutation	Rifampicin		Rifabutin		Reference
	MIC (μg /ml)	Fold increase [a]	MIC (μg /ml)	Fold increase	
M. leprae					
wild type	1		0.25		
GGC507→GGG (silent)	1	1	0.25	1	This study
GGC507→AGC (G507S)	0.5	0.5	0.125	0.5	(3)
ACC508→ACA (silent)	1	1	0.25	1	This study
CAG513→GTG (Q513V)	32	32	8	32	(3)
GAT516→AAT (D516N)	32	32	2	8	(14)
CAG517→CAT (Q517H)	1	1	0.25	1	(11)
CAC526→TAC (H526Y)	32	32	8	32	(14)
TCG531→TTG (S531L)	32	32	4	16	(3, 14)
TCG531→TGG (S531W)	32	32	8	32	(14)
GCG532→TCG (A532S)	1	1	0.25	1	(11)
CTG533→CCG (L533P)	32	32	4	16	(14)
GTC547→ATC (V547I)	1	1	0.25	1	This study
M. tuberculosis					
wild type	1	1	0.25		
GGC507→AGC (G507S)	0.5	0.5	0.125	0.5	(1)
GGC507→GAT (G507D)	0.5	0.5	0.125	0.5	(1)
ACC508→CAC (T508H)	0.5	0.5	0.125	0.5	(1)
ACC508→GCC (T508A)	1	1	0.25	1	(1)
CAG510→CAT (Q510H)	1	1	0.25	1	(22)
CTG511→CCG (L511P)	16	16	1	4	(1, 12)
CAA513→AAT (Q513N)	8	8	0.5	2	(1)
CAA513→GAA (Q513E)	32	32	2	8	(1)
GAC516→GAG (D516E)	8	8	0.5	2	(12)
GAC516→CAC (D516H)	1	1	0.25	1	(1)

369
370

371 Table 2 (continued) Rifampicin and Rifabutin susceptibilities of the recombinant *M.*
372 *smegmatis* strains

Mutation	Rifampicin		Rifabutin		Reference
	MIC (μg /ml)	Fold increase	MIC (μg /ml)	Fold increase	
GAC516→GTC (D516V)	32	32	2	8	(12, 21, 22)
CTG521→ATG (L521M)	1	1	0.125	0.5	(21)
TCG522→TTG (S522L)	>32	>32	8	32	(21)
GGG523→GCG (G523A)	1	1	0.125	0.5	(1)
GGG523→GGC (silent)	1	1	0.25	1	(1)
CAC526→CTC (H526L)	32	32	4	16	(12, 22)
CAC526→TAC (H526Y)	>32	>32	8	32	(12, 22)
CAC526→GAC (H526D)	>32	>32	8	32	(12, 22)
CAC526→TTC (H526F)	>32	>32	4	16	(1)
CAC526→AAC (H526N)	32	32	2	8	(8)
CAC526→CGC (H526R)	32	32	8	32	(12, 22)
CAC526→CAA (H526Q)	8	8	0.5	2	(1)
CGA529→AAA (R529K)	32	32	4	16	(22)
TCG531→TTC (S531F)	32	32	4	16	(1)
TCG531→TTG (S531L)	32	32	8	32	(21, 22)
506-508del [b]	16	16	0.5	2	(5)
514insTTC [c]	>32	>32	8	32	(12) (22)
518del [d]	32	32	2	8	(22)

373

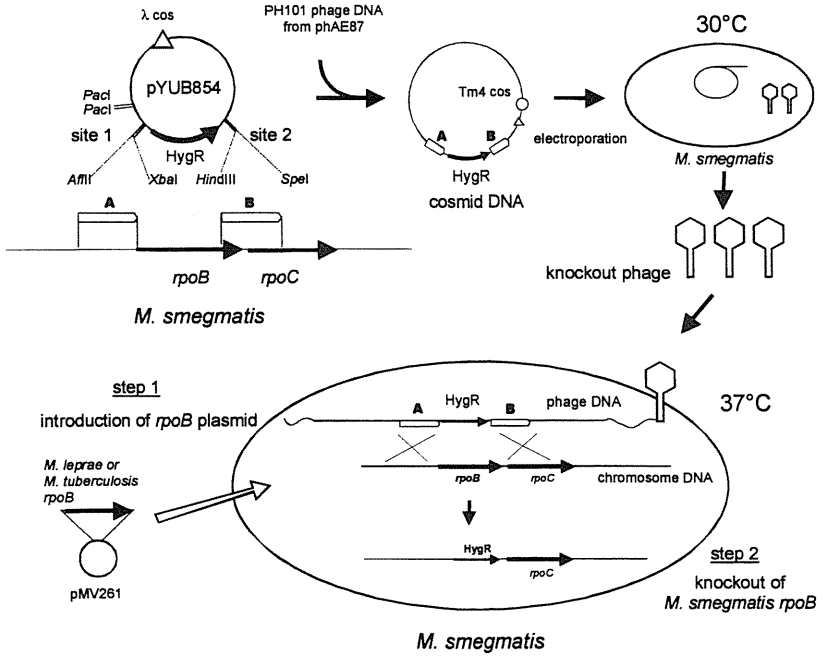
374 [a] fold increase in MIC compared to the wild type sequence, [b] deletion of codons 506-508, [c]
375 insertion of TTC between codons 514 and 515, [d] deletion of codon 518

376

377 Figure 1

378

379



380 Figure 2

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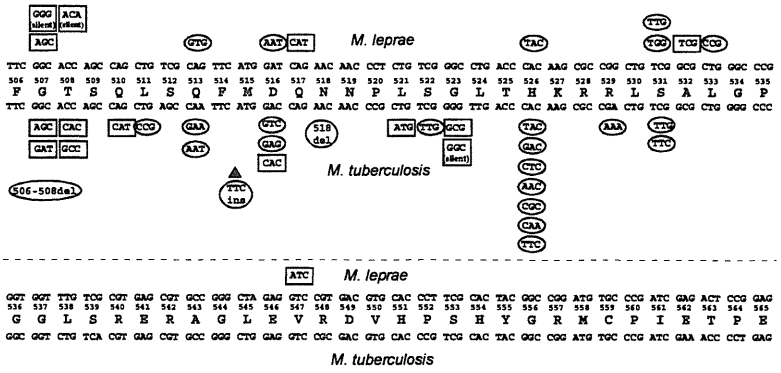

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Short Communication

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% ehtanol 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 visulized 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)[1)]	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)

[1)]: 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.

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Real-time PCR and high resolution melt analysis for rapid detection of *Mycobacterium leprae* drug resistance mutations and strain types

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Running title: *M. leprae* drug resistance screen and strain typing

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ABSTRACT

Drug resistance surveillance and strain typing of *Mycobacterium leprae* are necessary to investigate ongoing transmission of leprosy in endemic regions. To enable wider implementation of these molecular analyses, novel real time-PCR-high resolution melt (RT-PCR-HRM) assays without allele specific primers or probes and post PCR sample handling were developed. For the detection of mutations within drug resistance determining regions (DRDRs) of *folP1*, *rpoB* and *gyrA*, targets for dapsone, rifampicin and fluoroquinolones, real-time PCR-HRM assays were developed. A reference panel of wild type and drug resistant mouse foot pad derived strains which included three *folP1*, two *rpoB* and one *gyrA* mutation types were tested. RT-PCR-HRM correctly distinguished the wild type from the mutant strains. In addition, RT-PCR HRM analyses aided in recognizing samples with mixed or minor alleles and also a mislabeled sample. When tested in 121 sequence characterized clinical strains, HRM identified all the *folP1* mutants representing two mutation types, including one not within the reference panel. The false positives (<5%) could be attributed to low DNA concentration or PCR inhibition. A second set of RT-PCR HRM assays for identification of three previously reported SNPs that have been used for strain typing were developed and validated in 22 reference and 25 clinical strains. Real-time PCR-HRM is a sensitive, simple, rapid and high throughput tool for routine screening known DRDR mutants in new and relapsed cases, SNP typing and detection of minor mutant alleles in wild type background at lower costs than current methods and with potential for quality control in leprosy investigations.

INTRODUCTION