creative commons
COMMONS DEED

저작자표시-비영리-변경금지 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:

저작자표시. 귀하는 원저작자를 표시하여야 합니다.

비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.

변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 이용허락규약(Legal Code)을 이해하기 쉽게 요약한 것입니다.

Disclaimer

dCollection

연세대학교
YONSEI UNIVERSITY

Development of a droplet digital PCR method for detection and monitoring of *Mycobacterium tuberculosis* and multi-drug resistant tuberculosis

Yu Jeong Choi

Department of Medicine

The Graduate School, Yonsei University

연세대학교
YONSEI UNIVERSITY

연세대학교
YONSEI UNIVERSITY

Development of a droplet digital PCR method for detection and monitoring of *Mycobacterium tuberculosis* and multi-drug resistant tuberculosis

Yu Jeong Choi

Department of Medicine

The Graduate School, Yonsei University

연세대학교
YONSEI UNIVERSITY

Development of a droplet digital PCR method for detection and monitoring of *Mycobacterium tuberculosis* and multi-drug resistant tuberculosis

Directed by Professor Kyung-A Lee

The Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Medical Science

Yu Jeong Choi
December 2022

연세대학교
YONSEI UNIVERSITY

This certifies that the Doctoral Dissertation of
Yu Jeong Choi is approved.

[Signature]

Thesis Supervisor : Kyung-A Lee

[Signature]

Thesis Committee Member#1 : Yoonjung Kim

[Signature]

Thesis Committee Member#2 : Dokyun Kim

[Signature]

Thesis Committee Member#3: Young Ah Kim

[Signature]

Thesis Committee Member#4: Hye-Jung Park

[three signatures total in case of Master's]

The Graduate School
Yonsei University

December 2022

연세대학교
YONSEI UNIVERSITY

ACKNOWLEDGEMENTS

The completion of this study could not have been achieved if not for the support of my supervisor, Prof. Kyung-A Lee. I thank her deeply for the learning opportunity she has given me. I would also like to thank Prof. Yoonjung Kim, Prof. Dokyun Kim, Prof. Young Ah Kim, and Prof. Hye-Jung Park for taking the time to read my thesis and the constructive feedback they have given me. This thesis has improved greatly thanks to them.

I would like to express special thanks to Prof. Yoonjung Kim and Ms. Boram Choi for their guidance and expertise. None of this would have been possible without them.

To all my colleagues, friends and family for the support and encouragement they have shown me, I am forever grateful. Thank you.

Yu Jeong Choi

연세대학교
YONSEI UNIVERSITY

<TABLE OF CONTENTS>

연세대학교
YONSEI UNIVERSITY

LIST OF FIGURES

LIST OF TABLES

연세대학교
YONSEI UNIVERSITY

ABSTRACT

Development of a droplet digital PCR method for detection and monitoring of *Mycobacterium tuberculosis* and multi-drug resistant tuberculosis

Yu Jeong Choi

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Kyung-A Lee)

Background: The prevalence of MDR-TB among Korean tuberculosis patients is about 4.1%, which is higher than the OECD average of 2.6%. Inadequate drug use and poor patient compliance increase MDR-TB prevalence through selective pressure. Therefore, prompt detection of drug resistance in tuberculosis patients at the time of diagnosis as well as quantitative monitoring of these resistant strains during treatment is crucial.

Method: Multiplex ddPCR assay was developed and assessed using DNA material of nine *Mycobacterium tuberculosis* strains with known mutation statuses purchased from the Korean National Tuberculosis Association. We collected a total of 20 MDR-TB residual samples referred for PCR analysis. Total DNA and exosomal DNA were extracted and subjected to the quadruplex ddPCR assay. Their results were compared to the known resistance phenotypes.

Result: The LOB for *IS6110, katG, inhA, rpoB, embB, rrs, gyrA* and *rpsL* were 1, 0, 1.5, 0.5, 1.5, 0.5, 1.5, and 0 copies per reaction. No cross-reactivity was observed between mutant and wild type. The multiplex ddPCR results correlated well with phenotype but there were some discrepancies.

Conclusion: In conclusion, we have developed a sensitive and accurate multiplex ddPCR assay that can detect the presence of tuberculosis as well as resistance-conveying mutations concurrently. This tool could aid clinicians in the diagnosis and treatment of tuberculosis.

연세대학교
YONSEI UNIVERSITY

Key words : *Mycobacterium tuberculosis*, multidrug-resistant tuberculosis, multiplex ddPCR

연세대학교
YONSEI UNIVERSITY

Development of a droplet digital PCR method for detection and monitoring of *Mycobacterium tuberculosis* and multi-drug resistant tuberculosis

Yu Jeong Choi

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Kyung-A Lee)

I. INTRODUCTION

Pulmonary tuberculosis is a bacterial infection caused by *Mycobacterium tuberculosis*. It is highly contagious; about 30% of close contacts with tuberculosis patients get infected, and about 10% of these infected individuals develop pulmonary tuberculosis. According to the 2019 World Health Organization (WHO) Global Tuberculosis Report, over 10 million people fell ill with tuberculosis in 2018 and 1.5 million died of tuberculosis the same year worldwide[1]. Korea has the highest prevalence of tuberculosis among Organization for Economic Co-operation and Development (OECD) countries, with around 3 million newly diagnosed tuberculosis patients and 2 thousand deaths from tuberculosis every year as presented by the Korean Centers for Disease Control and Prevention (KCDC) on their annual report[2]. Moreover, the prevalence of multi-drug resistant tuberculosis (MDR-TB) among Korean tuberculosis patients is about 4.1%, which is higher than the OECD average of 2.6%[3]. In addition, inadequate drug use and/or poor patient compliance increases MDR-TB prevalence through selective pressure[4, 5]. Therefore, prompt detection of drug resistance in tuberculosis patients at the time of diagnosis as well as quantitative monitoring of these resistant strains during treatment is crucial.

The gold standard method to diagnose tuberculosis and detect its resistance is through bacterial culture[2]. However, since it takes 6 to 8 weeks to culture *Mycobacterium tuberculosis*, real-time RT-PCR methods are used to make quick diagnoses. The problem

연세대학교
YONSEI UNIVERSITY

is that the sensitivity of nucleic acid amplification methods is only half of that of the culture test[6, 7]. Specially in the case of smear-negative patients, who correspond to 20-50% of all tuberculosis patients, the sensitivity of real-time RT-PCR drops to 24.0% as opposed to 85.7% in smear positive patients[6]. A prior study reported that combining exosomal DNA with droplet digital PCR in such cases yielded a much-improved sensitivity of 64.0%[6]. This improvement can be contributed to the use of exosomal DNA and droplet digital PCR. Exosomes are small vesicles (30-100 nm) of endocytic origin that carry both host as well as pathogen-derived lipids, proteins, and nucleic acids[8]. Recent evidence has indicated that the exosomal nucleic acids are more stable than other forms of nucleic acids, probably by the protective effect of lipid bilayer coating[9]. They are abundantly found in various body fluids such as blood, sputum, urine, etc[10]. Changes in exosome composition and amount are currently being used as diagnostic markers and indicators of disease progression in cancer patients[11]. Cho S.M. et al. have shown that exosomes can be useful targets in tuberculosis patients, which is an intracellular pathogen[6].

Moreover, unlike the conventional way of detecting resistant strains, droplet digital PCR can measure the absolute nucleic acid count without using standard curves thanks to the production of droplets that allow PCR amplification of single template molecules. This procedure gives precise and reproducible data without the interference of PCR inhibitors that might affect conventional RT-PCR. Since 20,000 droplets are generated per sample, it has a very low limit of detection (0.005%)[12-14]. Based on such facts, we speculated that the combination of exosomal DNA with droplet digital PCR could be used to detect resistant strains and monitor therapeutic response with better sensitivity.

As for detecting resistance-conveying variants, Xpert MTB/RIF and Xpert MTB/XDR assays exhibit excellent sensitivity and specificity, even higher than the gold standard method of phenotypic drug susceptibility testing (pDST). Sensitivity and specificity of Xpert RIF at detecting rifampicin resistance was 92.7-95% and 98-99% respectively[15, 16]. Xpert XDR assay showed a sensitivity of 94-100% and a specificity of 100% for all drugs except ethionamide (specificity 97.3%) when compared to sequencing results[17].

연세대학교
YONSEI UNIVERSITY

Meanwhile, sensitivity of pDST ranged from 65.4% to 98.3% and specificity ranged from 95.0% to 99.7%[17]. These methods, however, are not fit for high-throughput analysis, which could be critical in laboratories dealing with large amounts of samples. Moreover, exact quantitation is not feasible, which could be important in the context of patient compliance or heteroresistance.

Our objective is to develop a new method that not only allows rapid diagnosis of tuberculosis with high sensitivity, but also enables detection of gene variants that confer drug resistance and monitor therapeutic response via quantitative measurement. To achieve this goal, we developed a droplet digital PCR-based panel that can detect the presence of tuberculosis and its drug resistance profile simultaneously. This was a proof-of-concept prototype, so we aimed to cover not all but the most prevalent resistance-conveying variants of each representative resistance gene.

As stated above, quick, and accurate diagnosis of tuberculosis, especially prompt recognition of drug resistance and compliance monitoring, are crucial in the proper management of tuberculosis. However, there is no method that can achieve all these goals. Real-time RT-PCR may be quick, but its sensitivity is only half of that of the culture test and lower in smear-negative patients[6, 7]. The culture test may be accurate, but it takes weeks, even months to yield a result. In addition, none of these methods are quantitative, which makes them unsuitable for follow-up and/or compliance monitoring. Since droplet digital PCR is a quantitative method and can detect drug resistance mutations of *Mycobacterium tuberculosis*, this combination could be the solution to the above-mentioned problems.

연세대학교
YONSEI UNIVERSITY

II. MATERIALS AND METHODS

1. Primers and probes

Primer-probe set for *IS6110* were as reported previously[6]. Regarding primers and probes targeting resistance genes (*rpoB*, *katG*, *inhA*, *embB*, *gyrA*, *rpsL*, and *rrs*), we did a thorough literature search to find the most common mutation responsible for the resistant phenotype for each gene (Supplementary table 1). Using those as our targets, primers and probes were designed with the Primer and Probes Design Tool offered by GenScript (Piscataway, New Jersey, USA). *InhA*, *rpoB*, *rpsL*, and *gyrA* probes were labeled with the fluorophore FAM, while *katG*, *rrs*, and *IS6110* probes were labeled with VIC. *EmbB* probes were labeled with HEX. Their sequences, concentrations, and attached dyes are summarized in Table 1.

연세대학교
YONSEI UNIVERSITY

Supplementary table 1 Summary of resistance mutations reported in prior studies

Study(yr)	RIF	INH		EMB	PZA	FQ	SM	KM	AMK
	rpoB	katG	inhA	embB	pncA	gyrA	rpsL	rrs	rrs
Jnawali *et al.* (2013) [19]	S531L(44.79) D516V(8.3) D516Y(13.54) H526Y(9.4)	S315T(30.2)	C(-15)T(21.4)	M306V(23.4) M306I(17.2) Q497R(7.8) Q497K(3.1)	L159R(8.3) T135P(3.6) D12A(2.6) H51P(2.1)	D94G(32.8) A90V(12.0) S91P(10.0)	K43R(12.5) K88R(4.7)	A1401G(7.3)	A1401G(7.3)
Ko *et al.* (2019) [18]	S450L(25.6) H445Y(2.6) R552L(2.6)	S315T(12.8) S315N(2.6) L378R(2.6) Y597D(2.6)	I21T(2.6) I25T(2.6) S94A(5.1)	M306V(7.7) M306I(5.1) M306L(2.6) Y319S(2.6) I419V(2.6)	S18Ter(2.6) T47P(2.6) H82Pfs(2.6) L85R(2.6)	A90V(5.1) D94G(2.6) D94N(2.6)	K43R(7.7) K88Q(2.6)		
Farhat *et al.* (2016)-J. Clin. Microbiol [22]						A90V(10.8) D94G(13.3) D94Y(2.1)			
Park *et al.* (2018) [20]	S450L(63.3)	S315T(93.3) Y113F(3.3)	Y113F(3.3)	M306V(16.7) M306I(20.0) Y319D(6.7)	A46E(6.7)	A90V(3.3) S91P(3.3)	K43R(20.0) K88R(6.6)		
Farhat *et al.* (2016) -Am J Respir Crit Care Med [21]	S450L(OR 70.0) D435V(OR 28.0)	S315T(OR 169.0)	C(-15)T(OR 18.5)	M306V(OR 14.2) M306I(OR 6.0) Q497R(OR 9.5)	H51R(OR inf)	D94G(OR 228.1) D94Y(OR inf) A90V(OR 126.8) D94A(OR inf)	K43R(OR 25.5) K88R(OR inf)	A1401G(OR 127.4)	A1401G(OR 222.9) A514C(OR 6.4)

INH, isoniazid; RIF, rifampin; EMB, ethambutol; PZA, pyrazinamide; FQ, fluoroquinolone; SM, streptomycin; KM, kanamycin; CAP, capreomycin; AMK, amikacin; OR, odds ratio

연세대학교
YONSEI UNIVERSITY

Table 1 Description of the primers and probes used in this study

Panel	Primer/probe	Sequence (5'->3')	Concentration (nmol/L)	Dye
1	IS6110-F	GGCGTACTCGACCTGAAAGA	450	
	IS6110-R	CTGAACCGGATCGATGTGTA	450	
	IS6110	ACCATACGGATAGGGGA	125	VIC
	rpoB-F	AGGAGTTCTTCGGCACCAG	900	
	rpoB-R	AGCCGATCAGACCGATGTT	900	
	rpoB450	CCGACTGTTGGCGC	250	FAM
	rpoB445	TTGACCTACAAGCGCCGA	250	FAM
	rpoB435	AATTCATGGTCCAGAACA	250	FAM
	inhA-F	GCTCGTGGACATACCGATTT	1800	
	inhA-R	CTTCAGTGGCTGTGGCAGT	1800	
	inhA(-15)	GGCGAGATGATAGGT	500	FAM
	katG-F	GGGCTGGAAGAGCTCGTAT	900	
	katG-R	CCGTACAGGATCTCGAGGAA	900	
	katG315	ATCACCACCGGCATC	250	VIC
2	embB-F	GTCGGACGACGGCTACATC	1800	
	embB-R	GCGGAAATAGTTGGACATGTAG	1800	
	embB306(V)	CCTGGGCGTGGCCCGAGTC	250	HEX
	embB306(I)	CCTGGGCATHGCCCGAGTCG	500	HEX
	rpsL-F	GCAGCGTCGTGGTGTATG	450	
	rpsL-R	CCTCGACCTGACTCGTCAAC	450	
	rpsL43	CTCCGAGGAAGCCG	125	FAM
	rrs-F	GTAATCGCAGATCAGCAACG	1800	
	rrs-R	CTCCCTCCCGAGGGTTAG	1800	
	rrs(1401)	CCCGTCGCGTCAG	500	VIC
	gyrA-F	AGACCATGGGCAACTACCAC	450	
	gyrA-R	GCTTCGGTGTACCTCATCG	450	
	gyrA94	TCTACGGCASCCTGG	125	FAM

연세대학교
YONSEI UNIVERSITY

2. Specimens

We received DNA material of nine Mycobacterium tuberculosis strains with known mutation statuses from the Korean National Tuberculosis Association, which were used for assay development and evaluation (Table 2). These included strains with mutations in *rpoB*, *katG*, *inhA*, *embB*, *gyrA*, *rpsL*, and *rrs* genes as well as two wild type strains. We also received eight strains of live Mycobacterium tuberculosis with known drug sensitivity phenotype, which were used to evaluate the performance of the assay.

Residual sputum samples pre-treated accordingly with NALC-NaOH for tuberculosis PCR (Gangnam Severance Hospital) and Xpert MTB/RIF assay (Sinchon Severance Hospital) were collected between 2019-2022. Among these, samples positive for mutation in the rifampicin resistance determining region (RRDR) of the rpoB gene were subjected to drug susceptibility testing. Residual samples from Gangnam Severance Hospital were also subjected to Xpert MTB/RIF assay retrospectively if more than 1.5 ml was left. Samples of patients that were initially culture-positive and did follow-up tests afterwards were included as well. We also collected samples from patients who had had a history of tuberculosis in the past. These clinical samples were used to compare assay results using total DNA and exosomal DNA.

Research involving human specimens complied with all relevant national regulations, institutional policies, and the tenets of the Helsinki Declaration (as revised in 2013). The study was approved by the Institutional Review Board of Severance Hospital, Seoul, Korea (IRB no. 3-2020-0455). The requirement for informed consent was waived.

연세대학교
YONSEI UNIVERSITY

Table 2 Mutation statuses of DNA material of nine Mycobacterium tuberculosis strains with known mutation statuses purchased from the Korean National Tuberculosis Association

No.	Susceptiblity phenotype	*katG*	*inhA*	*rpoB*	*embB*	*pncA*	*gyrA*	*rrs*	*rpsL*
16	pan-Susceptible	-	-	-	-	-	-	-	-
18	pan-Susceptible	-	-	-	-	-	-	-	-
9	Multidrug-Resistant	S315T R463L	-	D435V	M306V	D136N	D94G	-	-
46	Multidrug-Resistant	S315T R463L	-	S450L	Q497K	74 C → del	A90V	-	K43R
107	Multidrug-Resistant	S315T	-15 C → T	S450L	M306I G406D	L35P	-	-	-
2	Extensively drug-resistant	S315T R463L	-	D435V	-	C14W	-	1401 A → G	K43R
23	Extensively drug-resistant	R463L	-15 C → T	S450L	M306I	P62L	D94G	1401 A → G	-
32	Extensively drug-resistant	R463L	-15 C → T	S450L	M306L	G97S	A90V	1401 A → G	-
77	Extensively drug-resistant	S315T R463L	-	S450L	M306V	-	D94G	1401 A → G	-

연세대학교
YONSEI UNIVERSITY

3. DNA extraction

For total DNA analysis, 100 μL aliquots of the clinical specimens were re-suspended in DNA extraction buffer. Total DNA was extracted using QIAamp DNA mini kit (QIAGEN, Hilden, Germany) as stated in the manufacturer's instructions. For exoDNA analysis, exosomal fractions were isolated from 1 mL of clinical specimens with ExoQuick™ Exosome Precipitation Solution (System Biosciences Inc., Mountain View, CA, USA) as recommended by the manufacturer. The specimens were centrifuged at 3000 x g for 15 min to remove cells and cell debris. After adding 1/4 volume of ExoQuick Solution, they were refrigerated at 4℃ overnight. The mixture was centrifuged at 1500 x g for 30 min, and the supernatant was removed. Pellets were re-suspended in nuclease-free water. Subsequently, exosome-derived DNA was extracted using QIAamp DNA mini kits as described above.

4. ddPCR

Digital PCR reactions were performed with a QX200 Droplet Digital PCR System (Bio-Rad Laboratories, Hercules, CA, USA). The dPCR reaction mixture was composed of 10 μL dPCR Probe Supermix (Bio-Rad, California, US), 1800/900/450 nM primers per target, 500/250/125 nM probe per target, and 1 μL sample. The reaction mixture was topped up with ultrapure DNase- and RNase-free water to a final volume of 20 μL. Then, micro-droplets were generated from the mixture via QX200 Droplet Generator (Bio-Rad). The PCR mix was composed of 10 μL of Bio-Rad Super mix TaqMan, 0.9 μL of each amplification primer and 0.5 μL of each probe, and 0.5 μL of sample DNA. Results were analysed with QuantaSoft Analysis Pro software (Bio-Rad Laboratories). This provided the number of positive and negative droplets, as well as quantification of IS6110 of MTB, and resistance-related genes expressed as copies/uL of ddPCR reaction. At least two positive droplets were required for a positive test result of the ddPCR assay. The procedure of multiplex ddPCR is summarized in Figure 1 and the actual 2D plots of our assay are shown in Figure 2. Amplitude multiplex ddPCR method was used to distinguish different targets/target sets via unique clusters they formed.

연세대학교
YONSEI UNIVERSITY

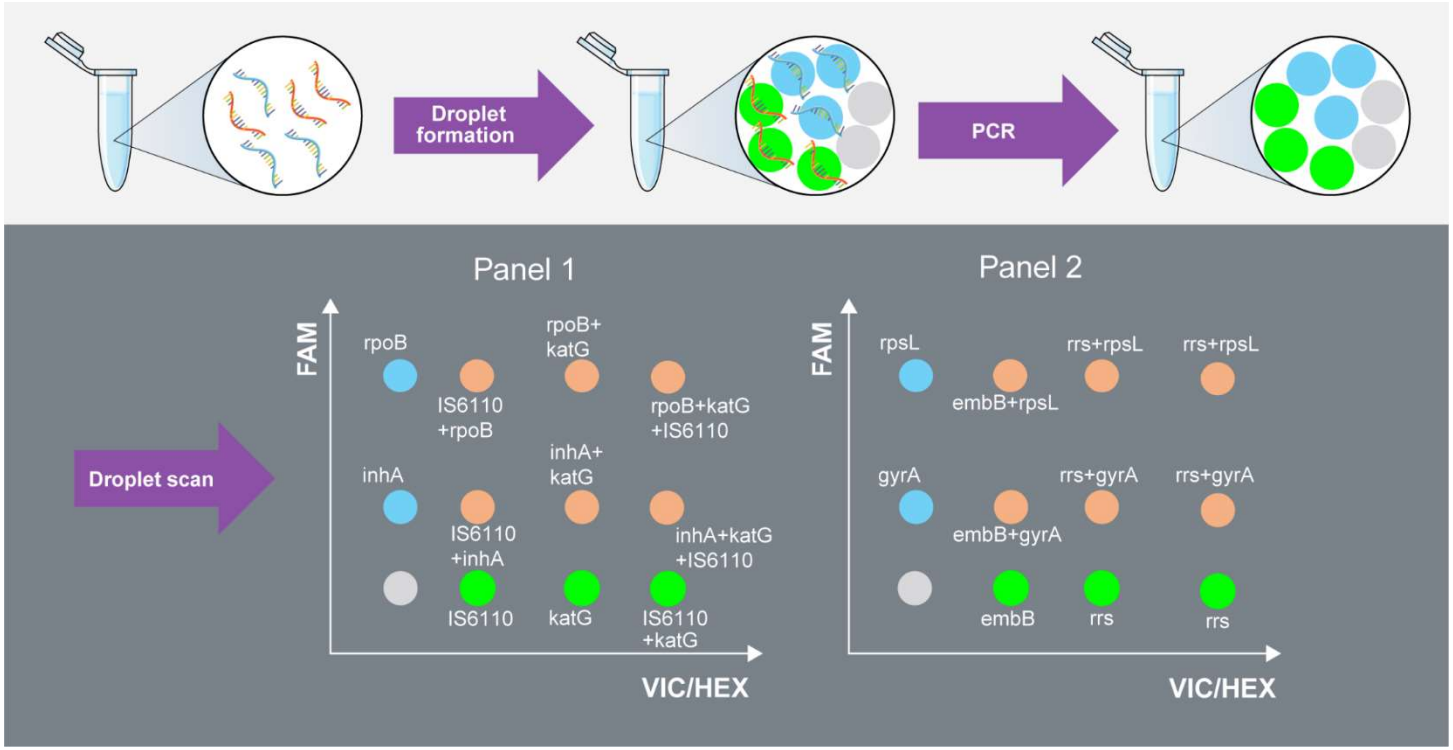
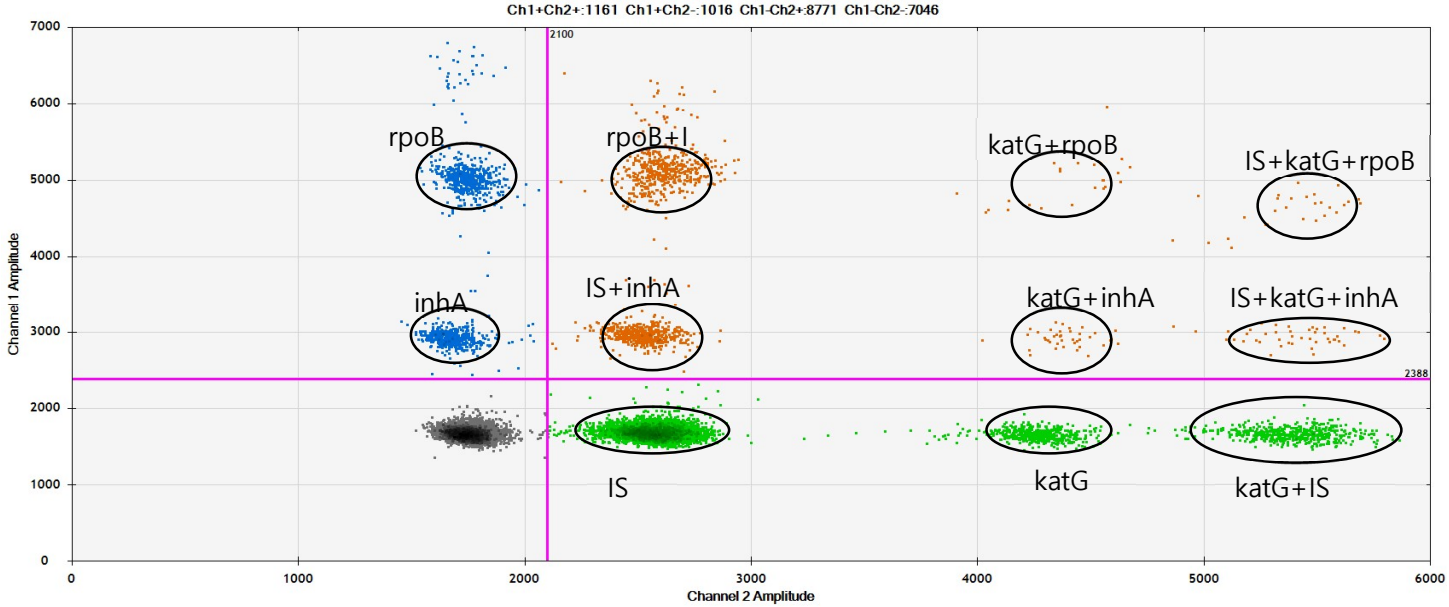


Figure 1 The sample mix undergoes droplet formation so that the DNA strands are compartmentalized into separate droplets. Then they are amplified via PCR and the amplified product reacts with fluorescence-labeled probe accordingly. Each droplet is scanned and plotted in its corresponding coordinate. The x-axis represents signal detected from Channel 2 (VIC/HEX) while the y-axis represents signal detected from Channel 1 (FAM).

연세대학교
YONSEI UNIVERSITY

(A)



연세대학교
YONSEI UNIVERSITY

(B)

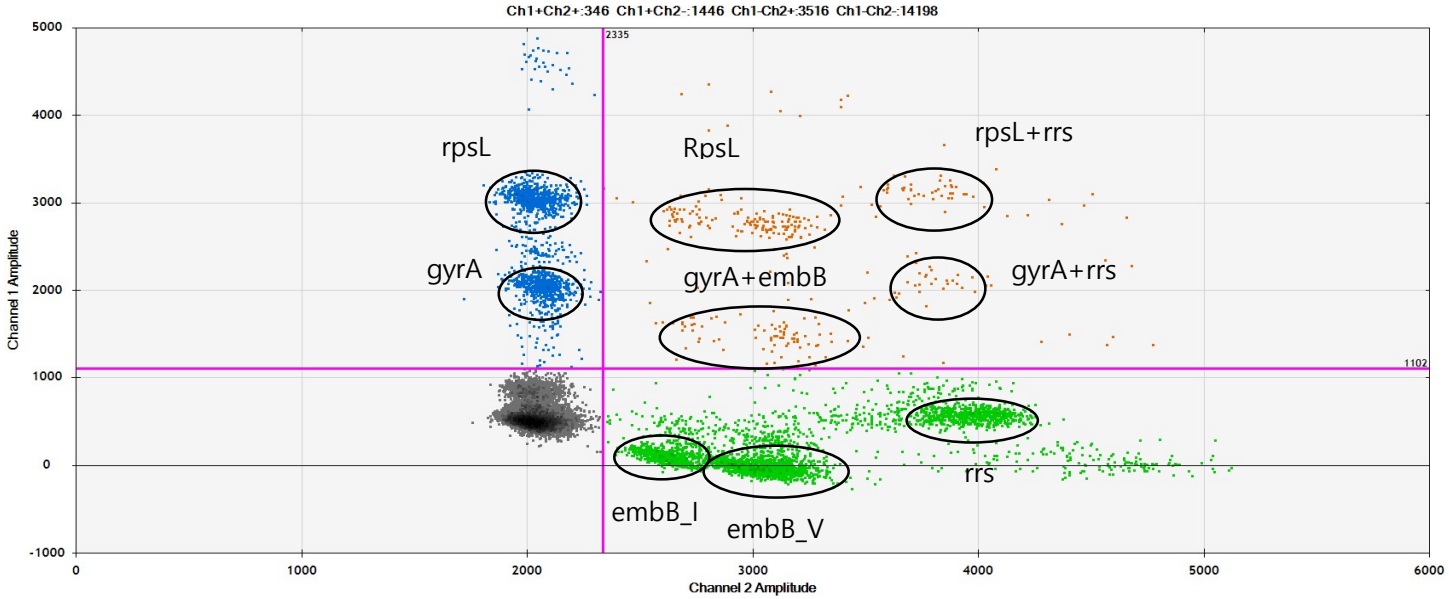


Figure 2 The 2D plots of panel 1 (A) and panel 2 (B) of our ddPCR assay is shown.

연세대학교
YONSEI UNIVERSITY

5. Performance evaluation of multiplex ddPCR

We designed two separate multiplex ddPCR assays to detect mutations on *rpoB*, *katG*, *inhA*, *embB*, *gyrA*, *rpsL*, and *rrs* as well as the *IS6110* gene specific to Mycobacterium tuberculosis complex (MTBC). *InhA*, *rpoB*, *katG*, and *IS6110* were put together as panel 1 while *embB*, *gyrA*, *rpsL*, and *rrs* were grouped as panel 2. To determine the limit of blank (LoB), twenty tuberculosis PCR negative sputum samples were tested. The assay's performance evaluation including sensitivity and specificity were validated as recommended[23]. Four DNA concentrations (50, 10, 5, 2 copies/μL) using g-block (IDT, Redwood City, CA, USA) were made for each corresponding probe to determine the limit of detection (LoD). The two higher concentrations (50, 10 copies/μL) were tested four times whereas eight replicates were tested in case of the lower two (5, 2 copies/μL).

6. Statistical analysis

For statistical analysis, Microsoft Excel 2013 (Seattle, WA, USA), Analyse-it for Microsoft Excel Method Evaluation Edition version 5.40.2 (Analyse-it Software, Ltd., Leeds, UK), SPSS Statistics v.23 (SPSS, Inc., Chicago, IL, USA), and Prism 8.0 (GraphPad Software, Inc, La Jolla, CA, USA) were used.

III. RESULTS

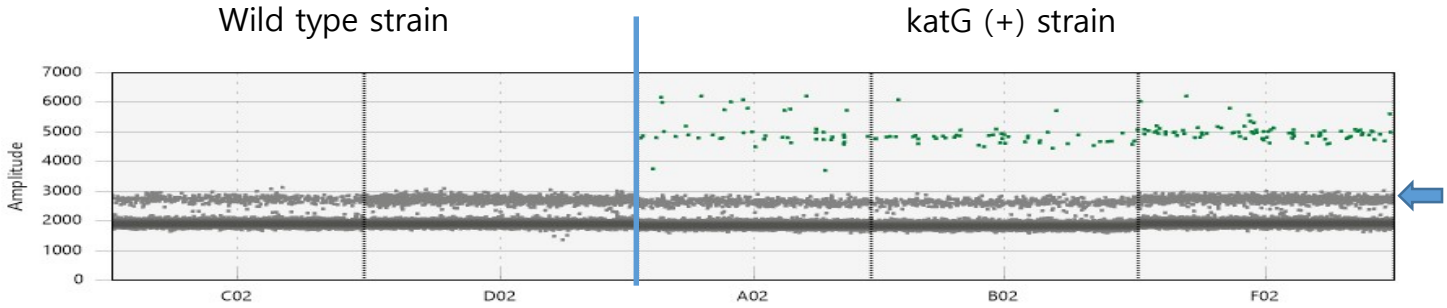
1. Performance of the multiplex ddPCR assay

The LoB for *IS6110*, *katG*, *inhA*, *rpoB*, *embB*, *rrs*, *gyrA* and *rpsL* were 1, 0, 1.5, 0.5, 1.5, 0.5, 1.5, and 0 copies per reaction. Thus, detection cut-off was set at 2 positive droplets. No cross-reactivity was observed between mutant and wild type (Supplementary figure 1). The LoD calculated by probit analysis for each probe was 3.09 copies/μL for rpoB_435, 2.97 copies/μL for rpoB_445, 2.83 copies/μL for rpoB_450, 2.97 copies/μL for inhA, 2.97 copies/μL for IS6110, 2.97 copies/μL for katG, 3.23 copies/μL for gyrA,

연세대학교
YONSEI UNIVERSITY

3.11 copies/μL for rpsL, 2.66 copies/μL embB_I, 2.86 copies/μL for embB_V, and 2.67 copies/μL for rrs.

연세대학교
YONSEI UNIVERSITY



Supplementary Figure 1 Result of multiplex ddPCR using 2 wild type strains (left) versus 3 strains containing *katG* mutation (right). The arrow indicates *IS6110*.

연세대학교
YONSEI UNIVERSITY

2. Multiplex ddPCR results of live *Mycobacterium tuberculosis*

The drug resistance phenotypes and genotype results obtained from multiplex ddPCR assays of eight live Mycobacterium tuberculosis are summarized in Table 3. Some discrepancies between known resistance phenotype and obtained resistance genotype were noted. T-3 was resistant to isoniazid, but no mutation was detected in neither *katG* nor *inhA*. T-2 and 6 were resistant to ethambutol while T-3 was sensitive but mutation on *embB* was only detected in T-3. All strains were sensitive to fluoroquinolones (ofloxacin and moxifloxacin) but mutation on *gyrA* was detected in T-5 and 7. Lastly, T-2 was resistant to streptomycin, but no mutation was detected on neither *rpsL* nor *rrs*.

연세대학교
YONSEI UNIVERSITY

Table 3 Resistance phenotype and ddPCR results of 8 live *Mycobacterium tuberculosis* strains

No.	Drug sensitivity test									ddPCR							
	INH	RIF	EMB	PZA	FQ	SM	KM	CAP	AMK	IS6110	katG	inhA	rpoB	embB	rrs	gyrA	rpsL
T-1	R	R	S	S	S	S	S	S	S	+	+	-	+	-	-	-	-
T-2	R	R	R	S	S	R	S	S	S	+	+	-	+	-	-	-	-
T-3	R	R	S	R	S	S	S	S	S	+	-	-	+	+	-	-	-
T-4	R	R	S	S	S	S	S	S	S	+	-	+	+	-	-	-	-
T-5	R	R	S	S	S	R	S	S	S	+	+	-	+	-	-	+	+
T-6	R	R	R	S	S	S	S	S	S	+	+	-	+	-	-	-	-
T-7	R	R	R	S	S	R	S	S	S	+	+	-	+	+	-	+	+
T-8	R	R	S	R	S	S	S	S	S	+	+	-	+	-	-	-	-

INH, isoniazid; RIF, rifampin; EMB, ethambutol; PZA, pyrazinamide; FQ, fluoroquinolone; SM, streptomycin; KM, kanamycin; CAP, capreomycin; AMK, amikacin

연세대학교
YONSEI UNIVERSITY

3. Multiplex ddPCR results of patient samples

The demographic characteristics of 20 samples obtained from 18 patients are summarized in Table 4. The mean age of the patients was 64.7 years and there was a male predominance. Regarding sample type, 85% were sputum samples while the other 15% were bronchial washing samples. In case of resistance phenotype, 55.6% of the patients harbored strains that were resistant to isoniazid and 38.9% were infected with rifampicin-resistant strains. Unknown resistance phenotypes were cases where either the culture was negative or contaminated or positive, but drug sensitivity test could not be done because the bacteria's activity was too low. All samples had a culture test done on the same day that molecular testing was done except for one sample, which was classified as "unknown". Positive culture test was found on 70% of the samples while 25% were culture negative and one sample, contaminated. AFB smear was negative in 70% of the samples.

연세대학교
YONSEI UNIVERSITY

Table 4 Demographic characteristics of 20 MDR-TB samples obtained from 18 patients

Variables	Patients/samples (n=18/20)
Age, years	
Mean (SD)	64.7/65.6 (14.6/14.4)
Sex	
Male (%)	11/12 (61.1/60)
Female (%)	7/8 (38.9/40)
Sample type (per sample)	
Sputum	17 (85)
Bronchial washing	3 (15)
Resistance phenotype (per patient)	
INH	10 (55.6)
RIF	7 (38.9)
EMB	3 (16.7)
PZA	3 (16.7)
FQ	0 (0.0)
SM	1 (5)
KM	0 (0.0)
CAP	0 (0.0)
AMK	0 (0.0)
Unknown	8 (44.4)
Culture (per sample)	
Positive	14 (70)
Negative	5 (25)
Contamination	1 (5)
AFB smear (per sample)	
3+	1 (5)
2+	4 (20)
1+	1 (5)
Negative	14 (70)

SD, standard deviation; AFB, Acid-fast bacillus

연세대학교
YONSEI UNIVERSITY

The multiplex ddPCR results of patient samples using total DNA as well as exosomal DNA are summarized in Supplementary tables 2 and 3. Similar discrepancies observed in those done with live strains were also seen with patient samples. Three culture negative samples tested positive for *IS6110* and 1 culture positive sample tested negative for *IS6110* using total DNA while 2 culture negative samples tested positive for *IS6110* and 1 culture positive sample tested negative for *IS6110* using exosomal DNA. No mutation was detected on 2 isoniazid-resistant samples on neither *katG* nor *inhA*, whereas mutation on *inhA* was found on one sensitive strain for both types of DNA. Similarly, no mutation on *rpoB* was observed in 3 and 4 rifampicin-resistant samples with total DNA and exosomal DNA respectively. For both types of DNA, 2 ethambutol-resistant strains tested negative for mutation. One fluoroquinolone-sensitive sample tested positive for *gyrA* mutation with exosomal DNA. Lastly, *rpsL* mutation was detected on 1 streptomycin-sensitive sample with total DNA while 1 streptomycin-resistant sample tested negative for mutation with both types of DNA. Results of the ddPCR assay using patient samples with known phenotypes as well as the eight strains received from the Korean National Tuberculosis Association and PCR negative samples were used to calculate the sensitivity and specificity when compared to pDST results (Table 5).

연세대학교
YONSEI UNIVERSITY

Supplementary table 2 Resistance phenotype of patient samples and their culture results

No.	Sample type	AFB smear	Sequencing		Tb Drug Susceptibility Test									Culture
			INH	RIF	INH	RIF	EMB	PZA	FQ	SM	KM	CAP	AMK	
G-1	Sputum	-	R	R	R	R	S	R	S	S	S	S	S	Positive
G-2	Sputum	-	R	R	R	R	R	R	S	S	S	S	S	Negative
G-3	Sputum	-	R	R	R	R	S	R	S	S	S	S	S	Positive
G-4	Sputum	-	R	S	R	S	S	S	S	S	S	S	S	Positive
G-5.1	Bronchial washing	2+	R	S	S	S	S	S	S	S	S	S	S	Positive
G-5.2	Sputum	2+	-	-	-	-	-	-	-	-	-	-	-	Positive
G-6.1	Sputum	-	R	S	S	S	S	S	S	S	S	S	S	Positive
G-6.2	Bronchial Washing	-	-	-	-	-	-	-	-	-	-	-	-	Negative
G-7	Sputum	-	R	S	S	S	S	S	S	S	S	S	S	Positive
S-1	Sputum	-	-	-	-	-	-	-	-	-	-	-	-	Positive
S-2	Sputum	3+	R	R	R	R	R	S	S	S	S	S	S	Positive
S-3	Sputum	1+	R	R	R	R	R	S	S	S	S	S	S	Positive
S-4	Sputum	2+	-	-	-	-	-	-	-	-	-	-	-	Positive
S-5	Sputum	-	-	-	-	-	-	-	-	-	-	-	-	Contamination
S-6	Bronchial washing	-	-	-	-	-	-	-	-	-	-	-	-	Negative
S-7	Sputum	-	-	-	-	-	-	-	-	-	-	-	-	Negative
S-8	Sputum	2+	-	-	-	-	-	-	-	-	-	-	-	Positive
S-9	Sputum	-	S	R	-	-	-	-	-	-	-	-	-	Positive
S-13	Sputum	-	-	-	-	-	-	-	-	-	-	-	-	Positive
S-14	Sputum	-	R	R	R	R	S	S	S	R	S	S	S	Negative

연세대학교
YONSEI UNIVERSITY

Supplementary table 3 Multiplex ddPCR results of patient samples

No.	IS6110		katG		inhA		rpoB		embB		rrs		gyrA		rpsL	
	Total	Exosome	Total	Exosome	Total	Exosome	Total	Exosome	Total	Exosome	Total	Exosome	Total	Exosome	Total	Exosome
G-1	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
G-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G-3	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-
G-4	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
G-5.1	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-
G-5.2	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G-6.1	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-
G-6.2	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-
G-7	+	+	-	-	+	+	-	-	-	-	-	-	-	+	-	-
S-1	+	+	-	-	+	-	+	+	-	-	-	-	-	+	-	-
S-2	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-
S-3	+	+	-	-	+	+	+	+	+	+	-	-	-	-	+	-
S-4	+	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-
S-5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S-6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S-7	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-
S-8	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-
S-9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S-13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S-14	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

연세대학교
YONSEI UNIVERSITY

Table 5 Sensitivity and specificity of samples tested with ddPCR assay

Drug	TP	FP	FN	TN	Sensitivity (%)	95% CI	Specificity (%)	95% CI
INH	15	0	1	29	93.75	69.77-99.84	100.00	88.06-100.00
RIF	11	0	1	33	91.67	61.52-99.79	100.00	89.42-100.00
EMB	2	1	3	39	40.00	5.27-85.34	97.50	86.84-99.94
FQ	0	2	0	43	-	-	95.56	84.85-99.46
SM	2	1	1	41	66.67	9.43-99.16	97.62	87.43-99.94
KM, CAP, AMK	0	0	0	45	-	-	100.00	92.13-100.00

TP, true positive; FP, false positive; FN, false negative; TN, true negative

연세대학교
YONSEI UNIVERSITY

The multiplex ddPCR results of follow-up patient samples using total DNA as well as exosomal DNA are summarized in Table 6. Sample types were consistent throughout the follow-up period. The DNA concentration of *IS6110* for total as well as exosomal DNA decreased progressively in all four follow-up cases.

Table 6 Multiplex ddPCR results of follow-up patient samples

No.	Date	Sample type	AFB smear	Culture	*IS6110* (copies/well)	
					Total	Exosome
1-1	2019-08-28	Sputum	3+	Positive	1667.45	289.55
1-2	2019-11-03	Sputum	Negative	Negative	9.43	2.86
2-1	2019-09-23	Sputum	Negative	Positive	21.32	1.55
2-2	2019-12-30	Sputum	Negative	Negative	1.46	0.00
3-1	2021-07-28	Sputum	3+	Positive	3028.48	1165.69
3-2	2021-09-24	Sputum	Negative	Positive	5.49	2.88
3-3	2021-11-10	Sputum	Negative	Negative	0.00	0.00
4-1	2021-10-22	Bronchial washing	Negative	Positive	45.90	3.37
4-2	2022-04-18	Bronchial washing	Negative	Negative	0.00	0.05

연세대학교
YONSEI UNIVERSITY

From the cycle threshold (Ct) value of each probe used in Xpert MTB/RIF assay, we could locate where in the RRDR the *rpoB* mutation was located. The mutation included in our ddPCR assay was covered by probe E according to the manufacturer. The comparison data is summarized in Supplementary Table 3. Of the nine samples collected from Gangnam Severance Hospital, only six had enough residual sample left for Xpert MTB/RIF assay. Among those six samples, only one sample had a mutation on the RRDR, probe E location. Droplet digital PCR result of this sample coincided with the Xpert MTB/RIF assay result. Samples S-5, 6, 7, 8, and 14 had mutations at locations other than probe E, which was corroborated by the fact that their ddPCR results were negative for *rpoB* despite a positive *IS6110*. All samples with mutations located on probe E were positive for *rpoB* mutation on ddPCR assay if *IS6110* was detected.

연세대학교
YONSEI UNIVERSITY

Supplementary table 3 Xpert MTB/RIF assay results and ddPCR results

No.	Sample type	AFB smear	Xpert		Probe	ddPCR	
			MTB	RIF		*IS6110*	*rpoB*
G-1	Sputum	Negative	Negative	Negative	-	+	-
G-2	Sputum	Negative	Negative	Negative	-	-	-
G-3	Sputum	Negative	Positive	Positive	probe E	+	+
G-4	Sputum	Negative	Positive	Negative	-	+	-
G-5.1	Bronchial washing	2+	Positive	Negative	-	+	-
G-5.2	Sputum	2+	Negative	Negative	-	+	-
G-6.1	Sputum	Negative	-	-	-	+	-
G-6.2	Bronchial washing	Negative	-	-	-	+	-
G-7	Sputum	Negative	-	-	-	+	-
S-1	Sputum	Negative	Positive	Positive	probe E	+	+
S-2	Sputum	3+	Positive	Positive	probe E	+	+
S-3	Sputum	1+	Positive	Positive	probe E	+	+
S-4	Sputum	2+	Positive	Positive	probe E	+	+
S-5	Sputum	Negative	Positive	Positive	probe B	-	-
S-6	Bronchial washing	Negative	Positive	Positive	probe B	-	-
S-7	Sputum	Negative	Positive	Positive	probe A	+	-
S-8	Sputum	2+	Positive	Positive	probe B	+	-
S-9	Sputum	Negative	Positive	Positive	probe E	-	-
S-13	Sputum	Negative	Positive	Positive	probe E	-	-
S-14	Sputum	Negative	Positive	Positive	probe B	+	-

연세대학교
YONSEI UNIVERSITY

IV. DISCUSSION

Overall, genotypic results of multiplex ddPCR assays correlated fairly well with the resistance phenotype. However, some exceptions were noted that need explaining. Regarding discrepancies found on multiplex ddPCR results of live *Mycobacterium tuberculosis*, Sanger sequencing of the same region as the probes used in the assays was done to confirm that the ddPCR results were indeed accurate sequence-level-wise. It is possible that they harbored another mutation in the same gene that was not covered by Sanger sequencing. One case was phenotypically resistant to isoniazid, but no mutation was detected on *katG* nor *inhA* (T-3). According to prior research, the most frequent form of discrepancy was genotypic susceptibility and phenotypic resistance to INH[24]. Authors of this paper speculated that rare mutations such as those in *kasA*, *msbA*, or, more commonly, *ahpC* could be the reason for such disparity. Discrepant result for *embB* was found on three out of eight strains: two were phenotypically resistant but genetically susceptible (T-2, T-6) while one was phenotypically susceptible but genetically resistant (T-3). Discordance in both ways has been reported by Ahmad et al., where the authors found that the agreement between phenotypic resistance and genotypic resistance was lowest for ethambutol: it was only 76% compared to 96% and 97% for rifampicin and isoniazid respectively[25]. *GyrA* mutation was detected in two fluoroquinolone-sensitive strains (T-5, T-7), though at very low concentrations: 6.32 and 3.30 copies per well. Heteroresistance can be one mechanism to explain this situation[26]. As for the *rpsL* mutation that was not detected in a strain resistant to streptomycin (T-2), mutation at another location could be the reason of discrepancy.

Regarding discrepancies found in the multiplex ddPCR results of patient samples, Sanger sequencing could not be done due to lack of remnant samples. However, discrepancies on drug resistance and ddPCR results were very similar to those found in live strains of *M. tuberculosis* except for G-1, which was resistant to rifampicin, but no mutation was detected on ddPCR. The most likely explanation is that the mutation is at a loci other than codons 450, 445, and 435 since Xpert RIF result was also negative for this sample

연세대학교
YONSEI UNIVERSITY

(Supplementary Table 3). One unique feature of the patient sample results was that the culture results could be compared with whether *IS6110* was detected or not. The discrepancy between them went both ways: there were samples that were culture negative but *IS6110* was detected and samples that were culture positive but no *IS6110* was detected. The former is sort of intuitive since ddPCR can detect *M. tuberculosis* dead or alive while culture tests only detect live bacteria. The latter, where culture is positive but molecular methods fail to detect *M. tuberculosis* is less intuitive, yet they have been reported before[27]. According to Nguyen et al., abnormal chest X-ray and low bacterial load in the sputum specimen were associated with both types of discordance.

Another interesting finding was that exosomal DNA was not superior to total DNA as was the case according to Cho et al.[6]. We speculate that while exosomal DNA enriched viable bacteria and thus correlated better with the culture test compared to total DNA when diagnosing tuberculosis infection, total DNA could be more appropriate in detecting resistance mutations, especially when they are MDR TBs that have endured treatment.

Sensitivity of ddPCR assay relative to pDST for INH was 93.75% (95% CI: 69.77–99.84) and specificity 100% (95% CI: 88.06–100), which were comparable to the sensitivity and specificity of Xpert MTB/XDR assays of 98.3% (95% CI: 95.8–99.3) and 95.0% (95% CI: 73.1–99.7) respectively[17]. In case of RIF, we yielded a sensitivity and specificity of 91.67% (95% CI: 61.52–99.79) and 100% (95% CI: 89.42–100), also comparable to the reported sensitivity of 81.0% (95% CI: 74.9–86.2) and specificity of 98.7% (95% CI: 93.0–100) for RIF using Xpert[15]. In case of FQ, KM, CAP, and AMK, the sensitivity could be calculated since there were no true positive nor false negative samples.

The fact that eight out of 18 strains with suspected resistance had unknown phenotypes was also noteworthy. Of those eight strains, one was too heavily contaminated by other bacteria, nothing grew in the culture test in two, and the viability of *M. tuberculosis* was too weak to carry out a drug sensitivity test in other two strains with a positive culture test. Since molecular methods can overcome these obstacles, multiplex ddPCR could aid in predicting resistance patterns in such cases.

연세대학교
YONSEI UNIVERSITY

Our research had several limitations. First, the number of samples collected were too small to draw a statistically meaningful conclusion. This was somewhat anticipated since the prevalence of MDR TB is only about 4% of all TB patients in Korea[3]. To make things worse, our sample collection period overlapped with the COVID-19 pandemic, during which the number of tuberculosis patients dropped either because less were infected thanks to the wearing of masks and thorough washing of hands or access to TB diagnostic services decreased and less were diagnosed regardless of the actual prevalence[28]. Another limitation was that due to the limited number of detection channels, no internal control could be added to our assay. Further study using more drug-resistant tuberculosis samples with known resistance phenotype could aid in assessing the utility of multiplex ddPCR more accurately.

V. CONCLUSION

In conclusion, we have developed a multiplex ddPCR assay that can detect the presence of tuberculosis as well as resistance-conveying mutations concurrently. This tool could aid clinicians in the diagnosis and treatment of tuberculosis.

연세대학교
YONSEI UNIVERSITY

REFERENCES

1. World Health Organization (WHO). Global Tuberculosis Report 2019. https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-report-2019 (Last accessed on January 2021)
2. Prevention KCfDCa. Korean Guidelines for Tuberculosis. In: Prevention KCfDCa, ed. 4th ed, 2020.
3. Cho KS. Tuberculosis control in the Republic of Korea. Epidemiol Health 2018;40:e2018036.
4. Zhang Y and Yew WW. Mechanisms of drug resistance in Mycobacterium tuberculosis. Int J Tuberc Lung Dis 2009;13:1320-30.
5. McGrath M, Gey van Pittius NC, van Helden PD, Warren RM, Warner DF. Mutation rate and the emergence of drug resistance in Mycobacterium tuberculosis. J Antimicrob Chemother 2014;69:292-302.
6. Cho SM, Shin S, Kim Y, Song W, Hong SG, Jeong SH, et al. A novel approach for tuberculosis diagnosis using exosomal DNA and droplet digital PCR. Clin Microbiol Infect 2020;26:942.e1-.e5.
7. Tostmann A, Kik SV, Kalisvaart NA, Sebek MM, Verver S, Boeree MJ, et al. Tuberculosis transmission by patients with smear-negative pulmonary tuberculosis in a large cohort in the Netherlands. Clin Infect Dis 2008;47:1135-42.
8. Rodrigues M, Fan J, Lyon C, Wan M, Hu Y. Role of Extracellular Vesicles in Viral and Bacterial Infections: Pathogenesis, Diagnostics, and Therapeutics. Theranostics 2018;8:2709-21.
9. Kahlert C, Melo SA, Protopopov A, Tang J, Seth S, Koch M, et al. Identification of double-stranded genomic DNA spanning all chromosomes with mutated KRAS and p53 DNA in the serum exosomes of patients with pancreatic cancer. J Biol Chem 2014;289:3869-75.
10. Huang T and Deng CX. Current Progresses of Exosomes as Cancer Diagnostic and Prognostic Biomarkers. Int J Biol Sci 2019;15:1-11.
11. Sheridan C. Exosome cancer diagnostic reaches market. Nat Biotechnol 2016;34:359-60.
12. Hindson BJ, Ness KD, Masquelier DA, Belgrader P, Heredia NJ, Makarewicz AJ, et al. High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. Anal Chem 2011;83:8604-10.
13. Dingle TC, Sedlak RH, Cook L, Jerome KR. Tolerance of droplet-digital PCR vs real-time quantitative PCR to inhibitory substances. Clin Chem 2013;59:1670-2.
14. Azuara D, Ginesta MM, Gausachs M, Rodriguez-Moranta F, Fabregat J, Busquets J, et al. Nanofluidic digital PCR for KRAS mutation detection and quantification in gastrointestinal cancer. Clin Chem 2012;58:1332-41.
15. Chakravorty S, Simmons AM, Rowneki M, Parmar H, Cao Y, Ryan J, et al. The New Xpert MTB/RIF Ultra: Improving Detection of Mycobacterium tuberculosis and Resistance to Rifampin in an Assay Suitable for Point-of-Care Testing. mBio 2017;8.

연세대학교
YONSEI UNIVERSITY

16. Dorman SE, Schumacher SG, Alland D, Nabeta P, Armstrong DT, King B, et al. Xpert MTB/RIF Ultra for detection of Mycobacterium tuberculosis and rifampicin resistance: a prospective multicentre diagnostic accuracy study. Lancet Infect Dis 2018;18:76-84.
17. Cao Y, Parmar H, Gaur RL, Lieu D, Raghunath S, Via N, et al. Xpert MTB/XDR: a 10-Color Reflex Assay Suitable for Point-of-Care Settings To Detect Isoniazid, Fluoroquinolone, and Second-Line-Injectable-Drug Resistance Directly from Mycobacterium tuberculosis-Positive Sputum. J Clin Microbiol 2021;59.
18. Ko D-H, Lee EJ, Lee S-K, Kim H-S, Shin SY, Hyun J, et al. Application of next-generation sequencing to detect variants of drug-resistant Mycobacterium tuberculosis: genotype–phenotype correlation. Annals of Clinical Microbiology and Antimicrobials 2019;18:1-8.
19. Jnawali HN, Hwang SC, Park YK, Kim H, Lee YS, Chung GT, et al. Characterization of mutations in multi- and extensive drug resistance among strains of Mycobacterium tuberculosis clinical isolates in Republic of Korea. Diagn Microbiol Infect Dis 2013;76:187-96.
20. Park J, Shin SY, Kim K, Park K, Shin S, Ihm C. Determining Genotypic Drug Resistance by Ion Semiconductor Sequencing With the Ion AmpliSeq™ TB Panel in Multidrug-Resistant Mycobacterium tuberculosis Isolates. Ann Lab Med 2018;38:316-23.
21. Farhat MR, Sultana R, Iartchouk O, Bozeman S, Galagan J, Sisk P, et al. Genetic Determinants of Drug Resistance in Mycobacterium tuberculosis and Their Diagnostic Value. Am J Respir Crit Care Med 2016;194:621-30.
22. Farhat MR, Jacobson KR, Franke MF, Kaur D, Sloutsky A, Mitnick CD, et al. Gyrase Mutations Are Associated with Variable Levels of Fluoroquinolone Resistance in Mycobacterium tuberculosis. J Clin Microbiol 2016;54:727-33.
23. Burd EM. Validation of laboratory-developed molecular assays for infectious diseases. Clin Microbiol Rev 2010;23:550-76.
24. Kang JY, Hur J, Kim S, Jeon S, Lee J, Kim YJ, et al. Clinical implications of discrepant results between genotypic MTBDRplus and phenotypic Löwenstein-Jensen method for isoniazid or rifampicin drug susceptibility tests in tuberculosis patients. Journal of Thoracic Disease 2019;11:400.
25. Ahmad S, Mokaddas E, Al-Mutairi N, Eldeen HS, Mohammadi S. Discordance across phenotypic and molecular methods for drug susceptibility testing of drug-resistant Mycobacterium tuberculosis isolates in a low TB incidence country. PLoS One 2016;11:e0153563.
26. Hofmann-Thiel S, Hoffmann H, Hillemann D, Rigouts L, Van Deun A, Kranzer K. How should discordance between molecular and growth-based assays for rifampicin resistance be investigated? Int J Tuberc Lung Dis 2017;21:721-6.
27. Nguyen HV, de Haas P, Nguyen HB, Nguyen NV, Cobelens FGJ, Mirtskhulava V, et al. Discordant results of Xpert MTB/Rif assay and BACTEC MGIT 960 liquid culture to detect Mycobacterium tuberculosis in community screening in Vietnam.

연세대학교
YONSEI UNIVERSITY

BMC Infect Dis 2022;22:506.
28. McQuaid CF, Vassall A, Cohen T, Fiekert K, White RG. The impact of COVID-19 on TB: a review of the data. Int J Tuberc Lung Dis 2021;25:436-46.

연세대학교
YONSEI UNIVERSITY

ABSTRACT(IN KOREAN)

결핵균 및 다제내성결핵의 검출 및 모니터링을 위한 드롭렛 디지털 PCR 방법 개발

<지도교수 이경아>

연세대학교 대학원 의학과

최유정

배경: 우리나라 결핵환자의 다제내성결핵 유병률은 약 4.1%로 OECD 평균인 2.6%보다 높다. 부적절한 약물 사용과 낮은 환자 순응도는 선택적 압력을 통해 다제내성 결핵 유병률을 증가시킨다. 따라서 결핵 환자의 약물 내성을 진단 당시 신속하게 감지하고 치료 중 이러한 내성 균주를 정량적으로 모니터링하는 것이 중요하다.

방법: Multiplex ddPCR assay는 대한결핵협회에서 구입한 돌연변이 상태가 알려진 9개의 Mycobacterium tuberculosis 균주의 DNA 물질을 사용하여 개발 및 평가하였다. PCR 분석이 의뢰된 총 20개의 MDR-TB 잔류 검체를 수집하였다. 전체 DNA 및 엑소좀 DNA를 추출하고 multiplex ddPCR 분석을 수행하였다. 그들의 결과는 알려진 내성 표현형과 비교되었다.

결과: IS6110, katG, inhA, rpoB, embB, rrs, gyrA 및 rpsL에 대한 LOB는 반응 당 1, 0, 1.5, 0.5, 1.5, 0.5, 1.5 및 0 카피였다. 돌연변이체와 야생형 사이에는 교차 반응성이 관찰되지 않았다. Multiplex ddPCR 결과 상 표현형과 좋은 상관관계가 있었지만 일부분 불일치가 관찰되었다.

결론: 결론적으로, 우리는 결핵의 존재와 내성 전달 돌연변이를 동시에 검출할 수 있는 민감하고 정확한 multiplex ddPCR 분석법을 개발했다. 이 도구는 임상의가 결핵 진단 및 치료에 도움이 될 수 있습니다.

핵심되는 말 : 결핵균, 다재내성 결핵, multiplex ddPCR