

# Comparison of REP13E12 PCR with Amplicor MTB for the Detection of *Mycobacterium tuberculosis* in Respiratory Specimens

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—Abstract—

**Backgrounds** : In recent years, the incidence of tuberculosis has increased mainly in high-risk populations. Classical laboratory diagnostic methods for tuberculosis have low sensitivity and time consuming procedures. Thus, it is important to identify the presence of *Mycobacterium tuberculosis* in the clinical specimens earlier than the culture results for the decision to initiate anti-tuberculosis therapy. Lee *et al.* reported a species-specific repeated sequence from a Korean *M. tuberculosis* isolate, which was later proved to be a part of REP13E12 repetitive sequence.

**Materials and Methods** : In this study, we compared the acid-fast staining, culture, Amplicor MTB (Roche), and REP13E12 PCR for detection of *M. tuberculosis* using 88 clinical samples. The sensitivity, specificity, positive and negative predictive values were compared.

**Results** : REP13E12 PCR showed equivalent score to Amplicor MTB. Both PCR-based methods showed better score than conventional stain and culture methods.

**Conclusion** : This result suggested that REP13E12 PCR is helpful for the rapid detection of the *M. tuberculosis* from clinical specimens.

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**Key Words** : *Mycobacterium tuberculosis*, Detection, REP13E12-PCR

Introduction

Tuberculosis is a major health problem  
that affects 8 million people and is responsible

for about 2 million deaths each year.<sup>1, 2)</sup> It is important to detect *M. tuberculosis* in clinical specimens quickly so that anti-tuberculosis drug therapy can be initiated. Several investigators have reported the PCR detection of *M. tuberculosis* DNA in clinical specimens<sup>3-10)</sup> Most of them were based on the IS6110 repetitive sequence.<sup>11)</sup> IS6110 is present in *M. tuberculosis* isolates with a copy number of 0-25. Thus, there are *M. tuberculosis* isolates without this repeated sequence. Lee *et al.* (GenBank U43540, 1995) reported a repeat sequence of 453 bp, from a Korean *M. tuberculosis* isolate, which is specific to the strains belonging to *M. tuberculosis* complex.<sup>12)</sup> In 1998, this repeat was proved to be a part of REP13E12 repetitive sequence from the genomic sequence data of *M. tuberculosis* H37Rv.<sup>13)</sup> In this study, we examined the usefulness of REP13E12-based polymerase chain reaction (PCR) by comparing with Amplicor MTB kit (Roche Molecular System, Branchburg, N.Y.), acid fast stain, and culture method for the detection of *M. tuberculosis* from clinical specimens.

## Materials and Methods

### Specimens and processing methods

Eighty-eight Korean clinical isolates from patients who were suspected to have pulmonary tuberculosis were obtained from Daegu-Gyungbuk Branch of the Korean Association of Tuberculosis. To minimize the

possibility of PCR contamination, DNA extraction, PCR amplification, and detection processes were carried out in separate rooms with different pipettes and tips with barriers. Equal volume of 4% NaOH was added to the specimen, vortex-mixed and incubated at room temperature for 15 min to decontaminate general bacteria. Two and a half volume of 0.067 M phosphate buffer (pH 6.8) was added and the mixture was centrifuged at 3,000 r.p.m. for 20 min. The decontaminated specimen was neutralized with 1 N HCl solution by dropping. These procedures were carried out within 24 hours after the specimen was received. Specimens were subjected to acid-fast stain, culture, REP13E12 PCR, and Amplicor MTB tests.

### Staining

Conventional Ziehl-Neelsen acid-fast staining method was used. AFB was reported on the basis of the following criteria at a 1000-fold magnification: no AFB seen, negative; one to nine per slide, few; one to nine per field, moderate; greater than nine per field, many.

### Culture

Each 100  $\mu$ l of decontaminated specimens were inoculated onto each of two Ogawa slants. The inoculated media were cultured 4-8 weeks at 37°C.

### REP13E12 PCR

DNA in the sputum specimens was prepared

as follows: decontaminated sputum pellets (200  $\mu\ell$ ) were mixed with 100  $\mu\ell$  of zirconium beads (0.1 mm in diameter), 100  $\mu\ell$  of distilled water, and 300  $\mu\ell$  of phenol-chloroform-isoamylalcohol (25: 24: 1, v: v). The mixture was shaken in a Mini-bead beater for 5 min and centrifuged at 10,000 x g for 10 min. The DNA in the aqueous phase was precipitated by addition of 2.5 volume of absolute ethanol. The DNA was dissolved in 30  $\mu\ell$  of TE (pH8.0) and used for PCR.<sup>14)</sup> Following primers were used for REP13E12 PCR; Forward: 5'-ACGTCAAAGTGATTCCGCG-3' and Reverse: 5'-CATGCCGTCGTATTGCTG -3'. The PCR mixture contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.1% Triton X-100, 0.5 mM of each primers, 200 mM of each of the 4 deoxyribonucleoside triphosphates (Boehringer Mannheim), 2.5 U of AmpliTaq (Perkin-Elmer Cooperation, Norwalk, Conn.), and appropriate amounts of DNA prepared from bacterial strains or clinical specimens.<sup>15)</sup> The PCR cycle was 5 min denaturation at 95°C, then 30 cycles (each cycle was 95°C for 1 min, 65°C for 1 min, and 72°C for 2 min), and a final extension cycle of 7 min at 72°C in a Perkin-Elmer 480 Cycler (Perkin-Elmer Cooperation, Norwalk, Conn.).

#### **Amplicolor MTB**

The Amplicolor MTB test was performed with instructions supplied by the manufacturer. A 100  $\mu\ell$  of each sample was mixed with

the wash solution and the mixture was centrifuged at 12,500 x g for 10 min. The supernatant was discarded and the pellet was resuspended with lysis reagent and incubated in a 60°C water bath for 45 min. The lysate was neutralized with 100  $\mu\ell$  of neutralization reagent provided with the kit. The processed specimen (50  $\mu\ell$ ) was mixed with a 50  $\mu\ell$  of master mixture solution containing biotin-labeled primers, nucleotides, and DNA polymerase. This system amplifies the 584 bp, 16S rRNA fragment of mycobacteria. The amplicon was chemically denatured and hybridized with probes specific for the *M. tuberculosis* complex. The biotin-labeled amplicons were identified by colorimetric detection (A450). A colorimetric reading exhibiting absorbance values greater than 0.35 optical density units was considered as positive.

#### **Results**

All 88 sputum specimens were examined by AFB stain, culture, REP13E12 PCR, and Amplicolor MTB. Of the 88 specimens, 12 were smear positive, 15 were culture positive, 16 were Amplicolor MTB positive, and 17 were REP13E12 PCR positive. The specimens divided into 9 groups as summarized in Table 1. Group I, positive in all of the tests; Group II, smear-negative and other tests positive; Group III, culture-negative and other tests positive; Group IV, smear- and

Table 1. Interpretation and grouping according to each test results

Group	Interpretation	No. of samples	AFB stain	Culture	Amplicor-MT	REP13E12
					B	PCR
I	Tb	9	+	+	+	+
II	Tb	2	-	+	+	+
III	Tb	1	+	-	+	+
IV	Tb	3	-	-	+	+
V	Tb*	1	+	-	+	-
VI	Tb*	2	-	+	-	+
VII	MOTT	1	+	-	-	-
VIII	MOTT	2	-	+	-	-
IX	None	67	-	-	-	-

Tb: *M. tuberculosis*, MOTT : mycobacteria other than tubercle bacilli, Group I to VI : *M. tuberculosis* positive, Group VII to IX : *M. tuberculosis* negative, \* : false positive,

culture- negative and REP13E12- and Amplicor MTB-positive; Group V, smear- and Amplicor MTB- positive and culture- and REP13E12- negative; Group VI, smear- and Amplicor MTB-negative and culture- and REP13E12 PCR-positive; Group VII, smear-positive and all other tests negative; Group VIII, culture-positive and all other tests negative; Group IX, negative in all of the tests. All tests

were duplicated.

Eighteen specimens that were positive at least two methods out of four (Group I to VI) was regarded these as golden positive standards. Thus, 70 samples were golden negative standards. Group VII and VIII, positive with only one method, were regarded as false positives (probably mycobacteria other than tubercle bacilli, MOTT). The

Table 2. Comparison of tests for detection of *M. tuberculosis* in clinical specimens

Method	Result	Sample No. of final interpretation		Sensitivity (%)	Specificity (%)	Predictive value(%)	
		Positive	Negative			Positive	Negative
Stain	Positive	11	1	61.1	98.6	91.7	90.8
	Negative	7	69				
Culture	Positive	13	2	72.2	97.1	86.7	93.2
	Negative	5	68				
Amplicor MTB	Positive	16	0	88.9	100	100	97.2
	Negative	2	70				
REP13E12 PCR	Positive	17	0	94.4	100	100	98.6
	Negative	1	70				

specimens that have the same test results with 4 test methods were 76 (77.3%).

The sensitivities, specificities, positive predictive values, and negative predictive values of each test were summarized in Table 2. The results showed that the sensitivities/specificities of AFB stain, culture, REP13E12, and Amplicor MTB were 61.1/98.6(%), 72.2/97.1(%), 94.4/100(%), and 88.9/100(%), respectively.

The positive predictive values of smear, culture, REP13E12, and Amplicor MTB were 91.7%, 86.7%, 100%, 100%, while the negative predictive values were 90.8%, 93.2%, 97.2%, 98.6%, respectively. Amplicor MTB and REP13E12 were more sensitive (88.9% and 94.4%) and specific (each 100%) methods than smear or culture methods for the detection of the *M. tuberculosis*. There were no statistically significant differences between sensitivities of Amplicor MTB and REP13E12.

#### Discussion

Both REP13E12 PCR and Amplicor MTB use PCR amplification of *M. tuberculosis*-specific DNA. However, REP13E12 PCR uses *M. tuberculosis*-specific primers while Amplicor MTB uses primers amplifying the DNA region encoding 16S rRNA that is common to all mycobacterial species, which was used for hybridization with *M. tuberculosis*-specific probes. This two-step procedure might explain the lower sensitivity found in

Amplicor MTB method, even though the results were not statistically significant.

AFB staining is very simple and rapid but has been known to be not sensitive. Usually AFB stain-positive is reported when more than ten thousands bacilli are present in specimens. The culture method showed 72.2% sensitivity and 97.1% specificity. The relatively low sensitivity of culture might be due to the complex procedures for decontamination which could kill *M. tuberculosis* bacilli in the specimens. The high sensitivity of amplification-based methods might be due to they uses DNA, which is very stable, not live bacilli for the tests.

The increased incidence of tuberculosis in not only underdeveloped countries but developed countries, due to the spread of human immunodeficiency virus (HIV) infection, has stimulated the need of rapid and direct detection methods for the laboratory identification of *M. tuberculosis*. Although numbers of investigators have reported PCR-based detection of *M. tuberculosis*, REP13E12 PCR is the only method that uses DNA sequence of a Korean *M. tuberculosis* isolate. The results of this study also suggested that PCR-based methods are simple, rapid, and acceptable for the detection of *M. tuberculosis* in clinical specimens.

#### 요 약

**배경** : 최근 결핵의 발병이 고위험군을 중심으로

로 증가하고 있다. 기존의 결핵 진단법은 오랜 시간이 걸리면서도 낮은 민감도를 가지고 있다. 따라서, 배양 결과 전에 빠른 치료 시작을 위해 임상 가검물에서 결핵균의 존재를 확인하는 것이 매우 중요하다. 이 등은 한국 결핵균 분리주로부터 종특이 반복서열을 발견하였고 이는 후에 REP13E12 반복서열임이 밝혀졌다.

**재료 및 방법** : 본 연구에서 저자는 88개의 임상가검물에서 결핵균을 검출하기 위해 항산성 염색, 배양, Amplicor MTB (Roche) 및 REP13E12 PCR법들을 비교하였다. 특히, 민감도, 특이도, 양성 및 음성 예측율을 비교하였다.

**결과** : REP13E12 PCR법은 Amplicor MTB법과 동등한 결과를 보였다. 이 두 가지 방법은 기존의 염색 및 배양법에 비해 보다 나은 성적을 보였다.

**결론** : 이 결과는 REP13E12 PCR법이 임상가검물에서 결핵균을 신속히 검출하는데 유용함을 시사하였다.

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