^{EZ}TB $_{PCR}$ FOR DIAGNOSIS of TUBERCULOSIS

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Introduction

1. Alarming increase of TB cases worldwide (WHO)-major global concern

2. MTB- most common causative of infection but infections by NTM are high in HIV patients

3. Treatment and management of MTB and NTM require different regimens of antibiotics and management methods

4. Current diagnostic tools: clinical features, microscopy (at least 10^5 organism/mL specimens to be visible) and culture (2-8 wks). Most important cannot differentiate between mycobacterium species

5. Urgent need of rapid identification and differentiation of mycobacterium for patients management and control of infections

Drawbacks in conventional PCR methods

(a) Most PCR detection method do not include internal control (IC) to rule out false negative results due to PCR inhibitors presence in clinical samples

(b) Requirement of cold-chain for reagents storage

(c) Procedures involved many pipetting steps- increase pipetting error and carry over contamination- leads to false positive results

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Initial work by Prof Zainul FZ (1988)- discovered IS986(1990- IS6110)

Developed with the Malaysian Bio-Diagnostic Research Group (MBDr)

Used to detect presence of both *Mycobacterium tuberculosis* and atypical mycobacterium in samples (sputum, CSF, Gastric, lavage, blood)

EZTB PCR Kit

Features and benefits

- convenient, simple, easy and fast to perform: requires few steps

-thermostabilized PCR mix: do not require ‘cold-chain’ for reagent storage

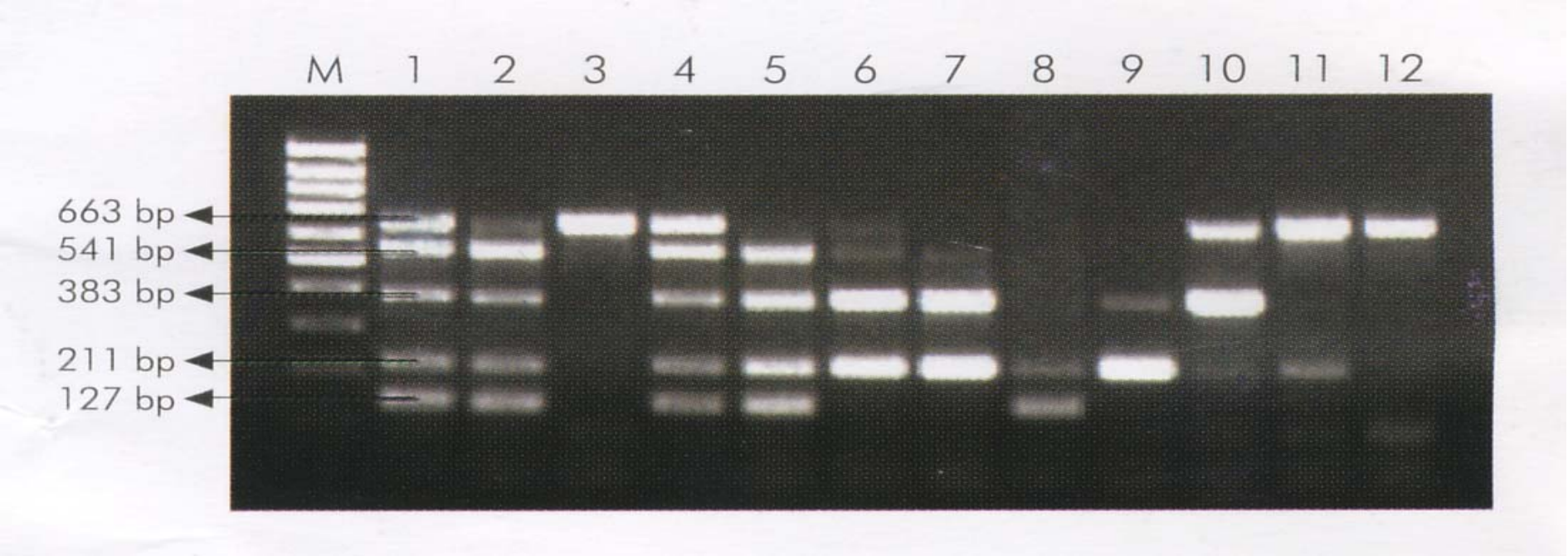
- ideal for private or hospital laboratories to screen varying number of tests

EZTB PCR Kit

Performance Characteristics

Method	: Multiplex PCR
Detection	: The presence of both *Mycobacterium tuberculosis* and atypical *Mycobacterium* using ***specific primers***
Specimens	: Sputum, Gastric lavage, CSF, Blood
Specimen volume	: 0.5- 1.0 mL of sample
Shelf life	: 9 months
Assay time	: 3 hrs
Detection level	: 1pg of Mycobacterial genomic DNA

Interpretation of results



Lane M: 100bp DNA marker, Lane 1: ^{EZ} TB $_{PCR}$ DNA ladder,
Lane 2: Positive control (MTB),
Lane 3: Negative control, Lane 4- Lane 8: Positive for M. tuberculosis,
Lane 9- Lane 11: Positive for atypical Mycobacterium
Lane 12: Negative for both M.tuberculosis and atypical mycobacterium

Summary of results interpretation

Amplicon size (bp)					Interpretation of the result
541	127	383	211	663	
+	+	+	+	+	Positive *M. tuberculosis* (Lane 4)
+	+	+	+	-	Positive *M. tuberculosis* (Lane 5)
+	-	+	+	+	Positive *M. tuberculosis* (Lane 6)
+	-	+	+	-	Positive *M. tuberculosis* (Lane 7)
-	+	-	+	-	Positive *M. tuberculosis* (Lane 8)
-	-	+	+	-	Positive *atypical mycobacterium* (Lane 9)
-	-	+	+	+	Positive *atypical mycobacterium* (Lane 10)
-	-	-	+	+	Positive *atypical mycobacterium* (Lane 11)
-	-	-	-	-	Both *M. tuberculosis* and atypical not detected

Evaluation of EZ TB PCR kit

Research Inst of Tropical Medicine, Alabang, Philipines	40 randomly selected pulmonary/ extrapulmonary specimens with known culture results	MTB: 100% sensitivity/specificity Atypical: 100% sensitivity, 90% specificity
University of Zimbabwe, Africa	13 cultured positive sputum	100% Sensitivity
University of Dacca, Bangladesh	28 cultured positive sputum	100% sensitivity
In-house evaluation (MBDr, Malaysia)	94 sputums samples from Philipines: 80 MTB cultured positive, 1 atypical cultured positive and 13 cultured negative samples	97.5% sensitivity 100% specificity

Conclusion

High sensitivity and specificity: allows identification of *M.tuberculosis*/ atypical mycobacterium infection

Thermostabilized PCR mix does not require freezer to store, convenient, simple, easy and fast to perform: ideal for private or hospital laboratories to screen varying number of tests per day

Thank you

EZ TB PCR kit