Evaluation of LAMP-TB system for detecting *Mycobacterium tuberculosis*

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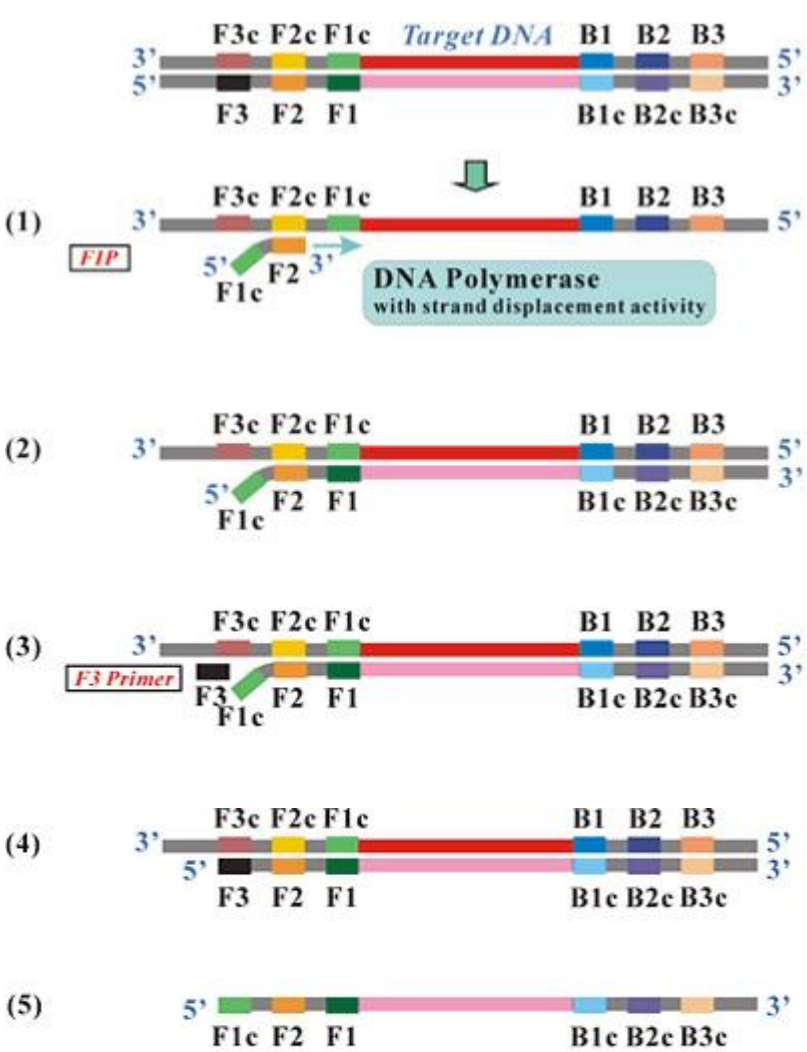
Introduction

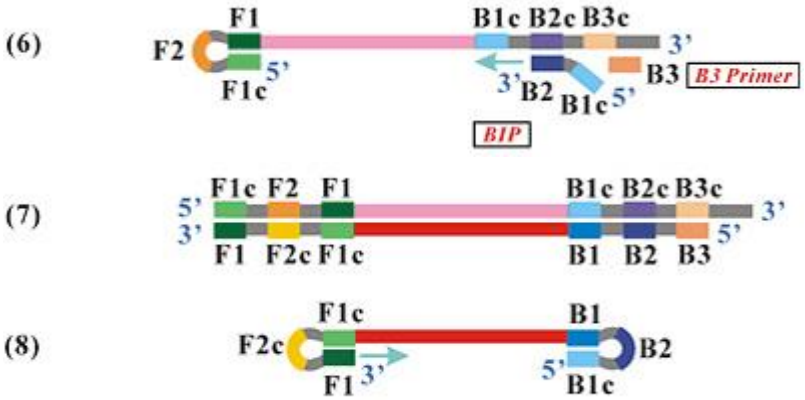
- Laboratory tests are crucial for tuberculosis control.
  - Detection, treatment monitoring & drug susceptibility testing
- Limitations of conventional tests for TB
  - Smear microscope: low sensitivity & specificity
  - Culture: complex & long TAT
- Limitations of current molecular tests
  - Require facilities & equipment
  - Require technical expertise & human resources
  - Often not feasible in resource-poor settings or remote area

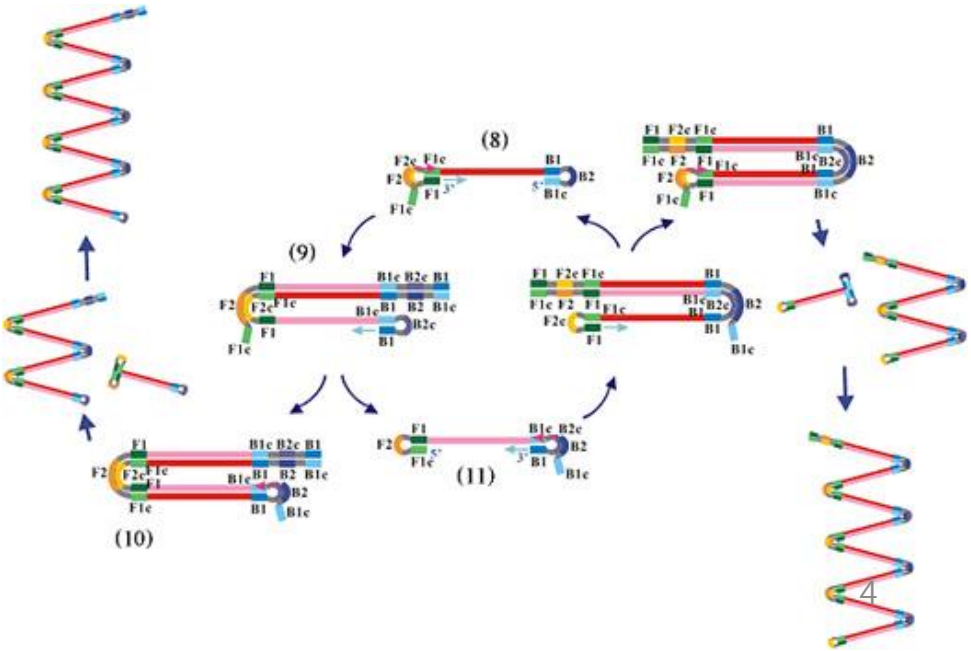
Introduction

- Loop-mediated isothermal amplification (LAMP)
  - Simple and rapid nucleic acid amplification method
  - Using 4 primers for 6 regions and one enzyme
  - Isothermal amplification
- LAMP-TB system
  - Detecting MTB using LAMP method in clinical sample
  - Reaction time: ~60 mins
  - Simple DNA extraction kit / Premix reaction tube
  - Mitarai et al.: sensitivity - 98.2% for Sm(+)/Cx(+), 55.6% for Sm(-)/Cx(+)

Introduction-LAMP







Global TB diagnostic pipeline

	Early Development	Late or Completed Development	On Pathway to WHO Evaluation
HIGH COMPLEXITY ASSAYS	**Molecular Detection/DST**		
	TruArray MDR-TB (Akkoni) COBAS TaqMan MTB +DST(Roche) Hydra 1K (insilixa) Mycobacterium Real-time MDR (CapitalBio)	TRC Rapid MTB (Tosoh) VereMTB (Veredus Laboratories) LiPA Pyrazinamide (Nipro) LATE-PCR Lights on / Lights off (Hain) TBMDx (Abbott) Meltpro (Zeesan) Mycobacteria RT PCR (CapitalBio) REBA MTB-XDR (YD Diagnostics) EasyNAT TB (Ustar) BD Max (BD)	GenoTYPE MTBDRsl (Hain) LiPA MDR-TB (Nipro) REBA MTB-Rifa (YD Diagnostics)
	Culture-based Technology		
	BNP Middlebrook (NanoLogix) Rapid colorimetric DST	TREK Sensitive MYCOTB (Trek)	
MODERATE COMPLEXITY ASSAYS	**Molecular Detection/DST**		
	Xpert Ultra and Xtend XDR (Cepheid) Alere Q (Alere) Enigma ML (Enigma Diagnostics) Q-POC (QuantuMDx) EOSCAPE (Wave80) RT-PCR Testing Platform (NWGHF/Guidel) iCubate 2.0 (iCubate) TBDx system (KGI) DiagCORE (STAT Diagnostica) LabChip G2-3 (Nanobiosys)	Genedrive MTB/RIF (Epistem) Truelab/Truenat MTB (Molbio)	TB LAMP (Eiken)
	Volatile Organic Compounds		
	BreathLink (Menssana) Prototype breathanalyzer (Next Dimensions) TB Breathalyser (Rapid Biosensor Systems) Aeonose (The eNose Company) Breath analysis instrument (Metabolomx)	Giant African Pouch Rats (Apopo)	
	Automated Microsvopy & Imaging		
	TBDx (Applied Visual Sciences) Fluorescent microscopy (ID-FISH Tech.) Automatic TB Screener (Fluorobot)	Microimager (BD) CAD4TB (Delft Imaging Systems)	

Purpose of the study

- Evaluate the performance of LAMP-TB system for detecting TB comparing with conventional tests and real-time PCR

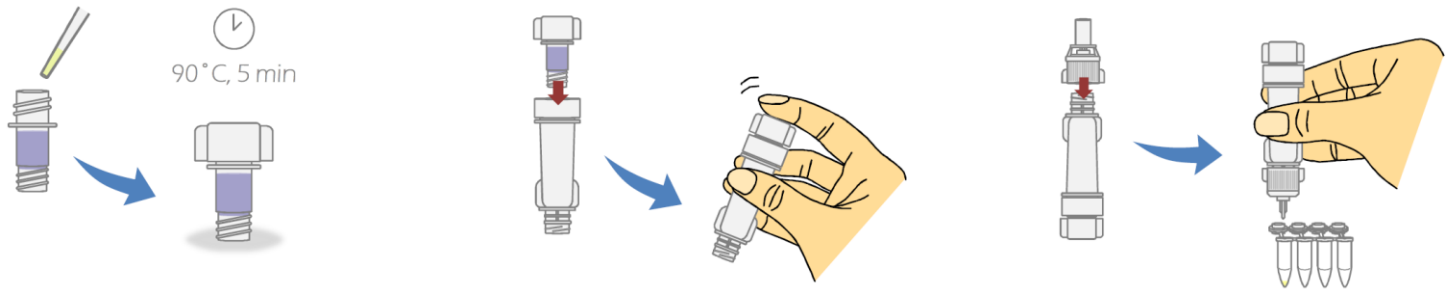
Materials and Methods

- **Specimen**: 226 sputum samples from TB suspects of health centers
- **Conventional tests**
  - Smear microscope: auramine O with WHO grading
  - Culture: solid culture (2% Ogawa), liquid culture (MGIT 960)
- **Real-time PCR**
  - DNA extraction: heat-extraction
  - Reagent: Advansure MTB/NTM kit (LG lifescience, Korea)
- **LAMP-TB**
  - DNA extraction: Loopamp™ Pure DNA extraction kit
  - Reagent: Loopamp™ MTBC detection kit
  - Amplification and detection: LA-500 (Eiken, Japan)

DNA Extraction

(Loopamp™ PURE DNA Extraction Kit)

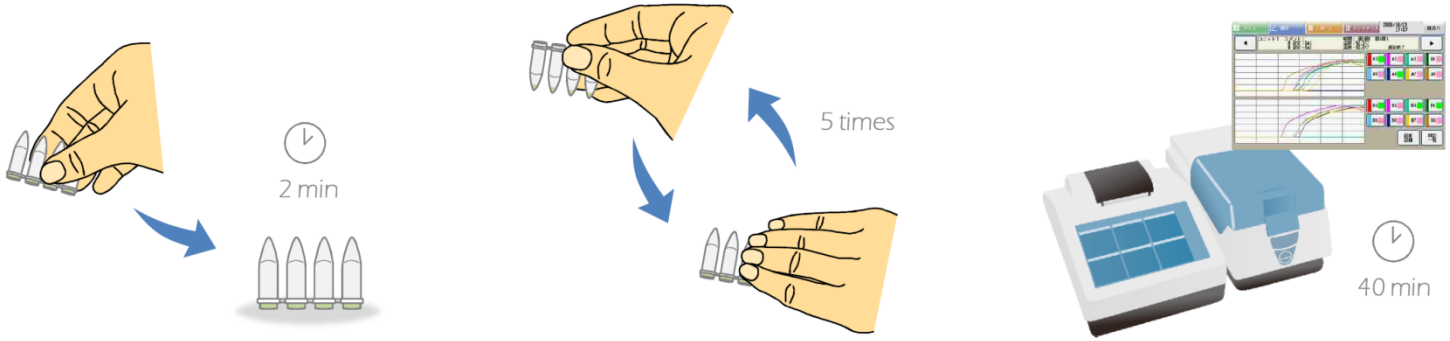
1. Pipette sputum into Heating Tube and incubate at 90˚C for 5 minutes
2. Connect Heating Tube to Adsorbent Tube and mix completely by shaking
3. Attach Injection Cap. Squeeze and drop sample into reaction tube



Amplification and Detection

(Loopamp™ MTBC Detection Kit)

1. Invert reaction tubes to collect solution in cap. Leave tubes standing upside down for 2 minutes
2. Mix solution with inversion of tubes and spin down tubes
3. Set reaction tubes to turbidimeter or incubator and start LAMP reaction



Culture vs LAMP-TB

Culture	LAMP-TB		Total
	Negative	Positive	
MTB	4 (26.7%)	11 (73.3%)	15
NTM	33 (100%)	-	33
No growth	154 (97.5%)	4 (2.5%)	158
Contamination	1 (100%)	-	1
Pending	2 (10.5%)	17 (89.5%)	19
Total	**194 (85.8%)**	**32 (14.2%)**	**226**

Sm(+)/Cx(+) = 87.5% (7/8), Sm(-)/Cx(+) = 57.1% (4/7)

LAMP-TB results according to Smear grade

Smear results	Positivity of LAMP-TB	Total
Negative	5 (3.2%)	157
1+	18 (72.0%)	25
2+	5 (71.4%)	7
3+	4 (100%)	4
Total	32 (16.6%)	193
Smear positive	27 (75.0%)	36

※ 33 samples which were culture positive for NTM were not included.

LAMP-TB vs Real-time PCR

Real-time PCR	LAMP-TB		Total
	negative	Positive	
MTB	6 (15.8%)	32 (84.2%)	38
MIX	1 (100%)	-	1
NTM	33 (100%)	-	33
Negative	154 (100%)	-	154
Total	**194 (85.8%)**	**32 (14.2%)**	**226**

Correlation between real-time PCR ct value & LAMP-TB

ct value	LAMP-TB		Total
	Positive	Negative	
<30	30 (96.8)	1 (3.2)	31
≥30	2 (25.0)	6 (75.0)	8
Total	32 (82.1)	7 (17.9)	39

Conclusions

- LAMP-TB system is easy to perform with very low sample volume.
- Sensitivity and specificity of LAMP were similar with previous studies.
- Sensitivity of LAMP is less than that of real-time PCR.
- No. of samples positive for MTB was not enough for evaluation. The further study is needed.