

Rapid detection of *Mycobacterium tuberculosis* complex from sputum samples using novel Loop-Mediated Isothermal Amplification

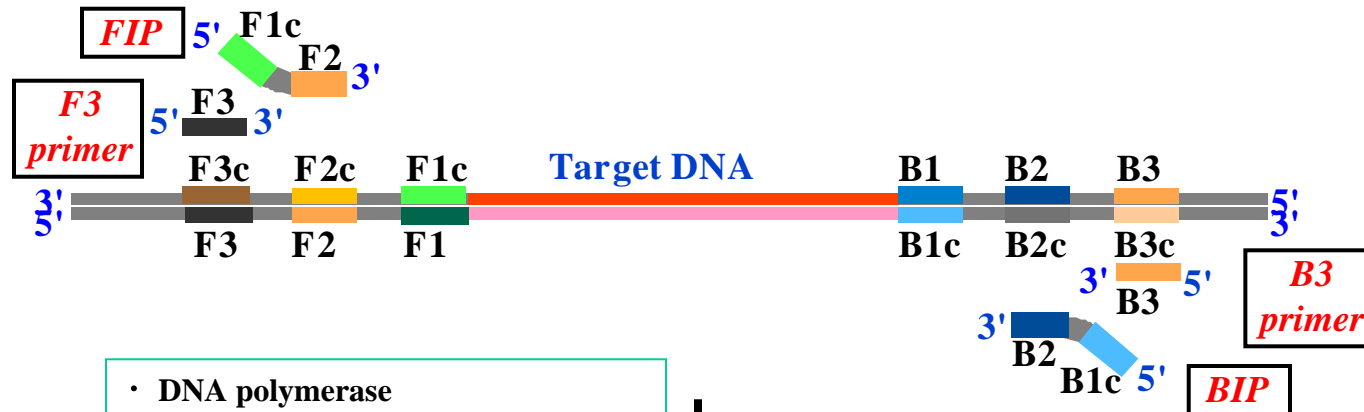
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EIKEN CHEMICAL Co.,LTD.

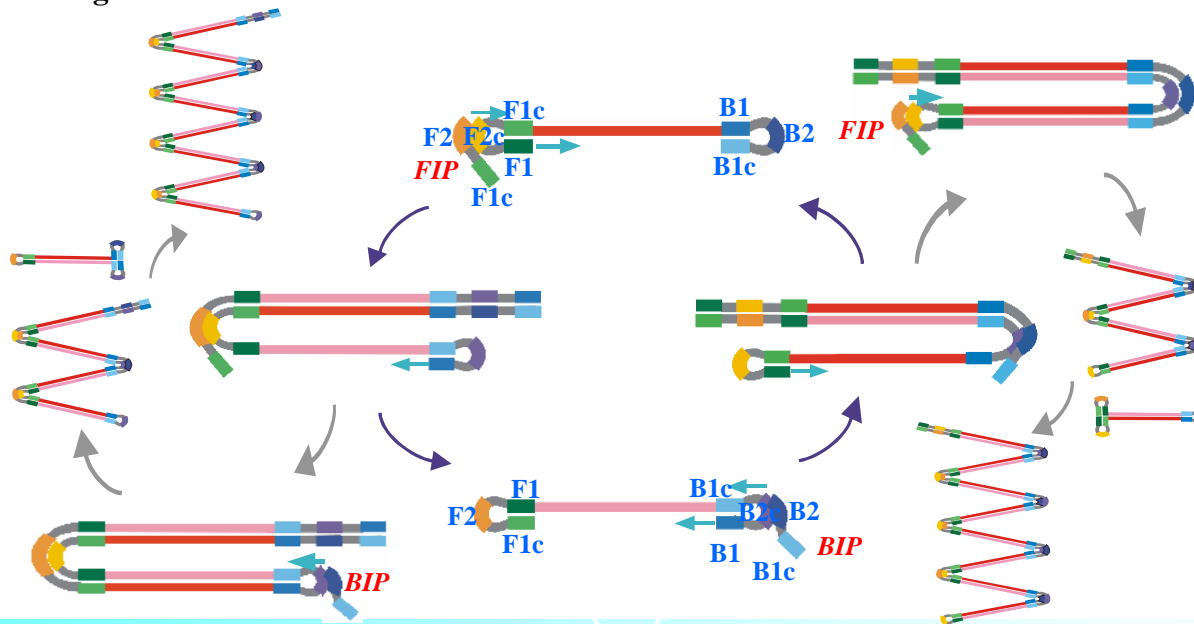
Basic principle of LAMP method

Mechanism of LAMP reaction



- DNA polymerase (with strand displacement activity)
- dNTPs
- Mg^{++}

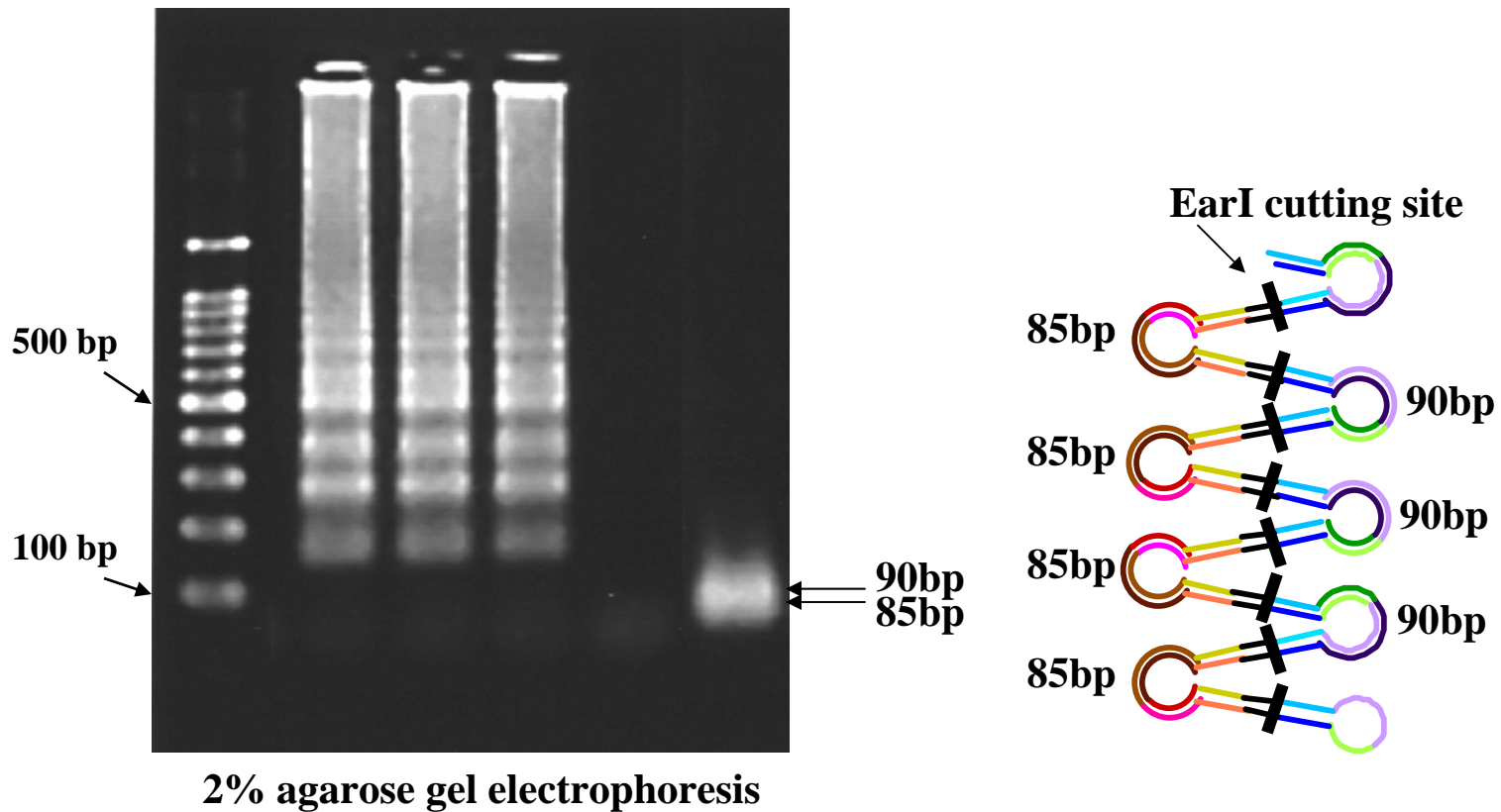
Incubate at 60°C ~ 65°C



Products of LAMP reaction

HBV

(copies/reaction) M 600 60 6 0 Ear I



Procedure of LAMP method

- Target DNA
- Primers (FIP, F3, BIP, B3)
- DNA Polymerase with strand displacement activity
- dNTPs
- Buffer solution

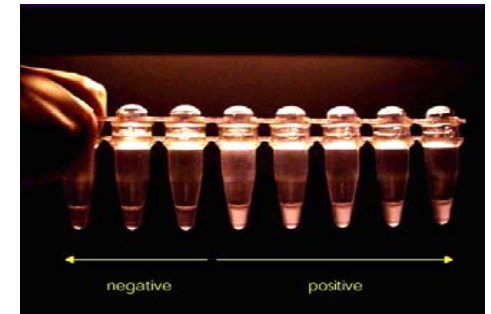
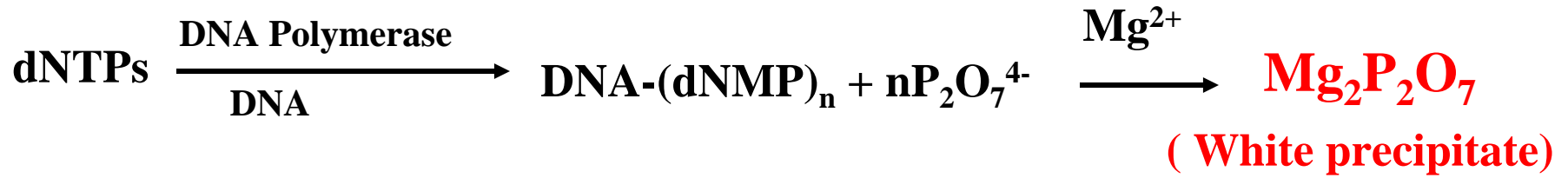


60 – 65C
15 - 60 min.

Detection

- **Simple**
- **Rapid (high efficiency)**
- **Specific (high specificity)**
- **Cost effective**

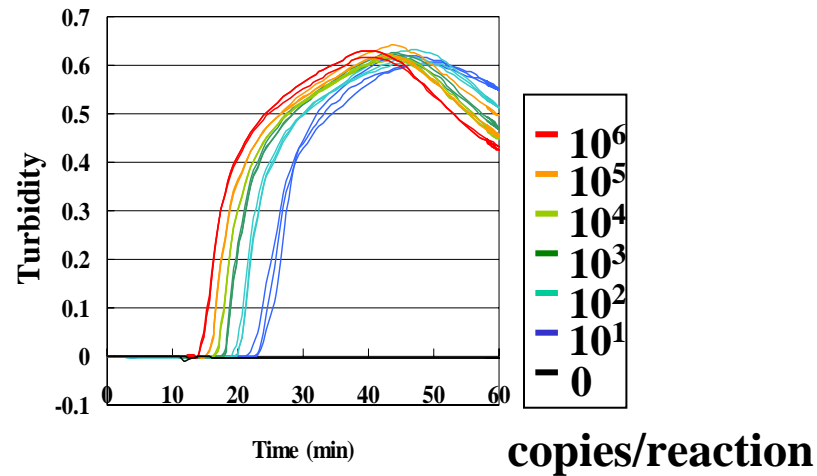
Detection -Turbidity -



<Real time monitoring>

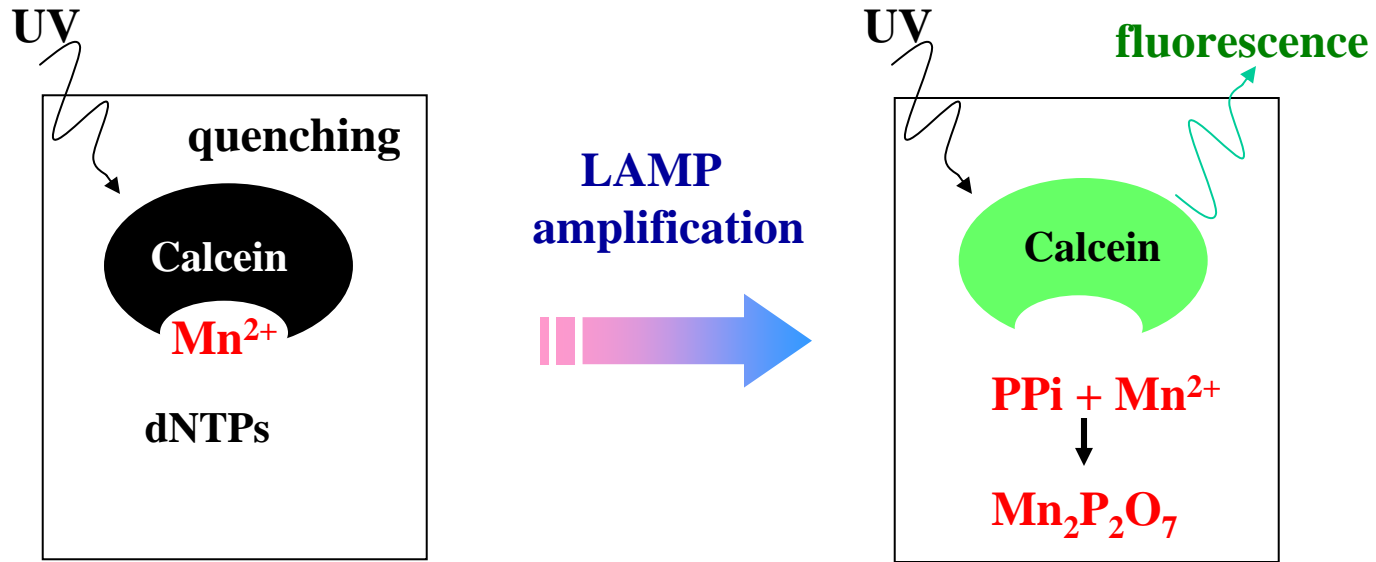


Real-time turbidimeter
LA200



Purified HBV genomic DNA was used as a template.

Detection - visible fluorescence -



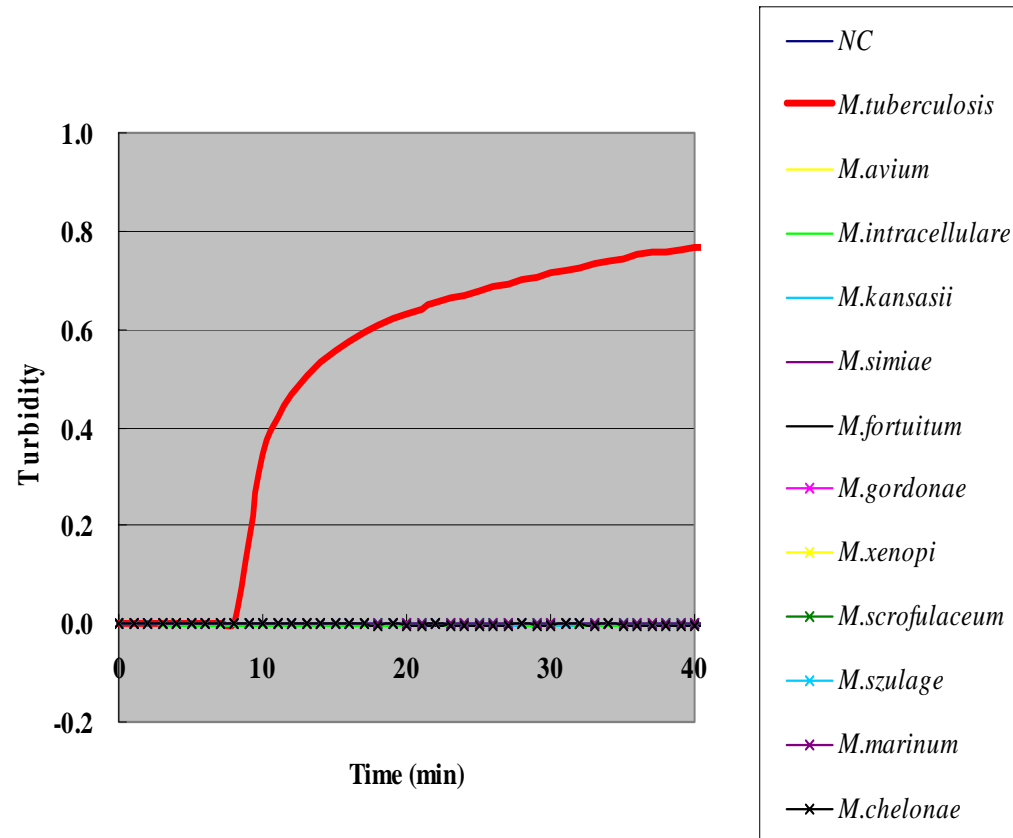
<Instrument for the detection>



- + (TB)

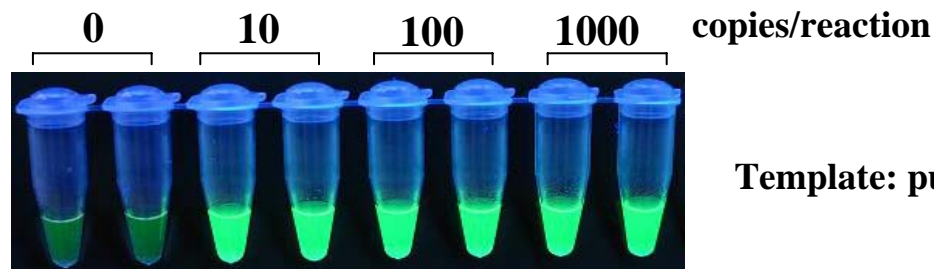
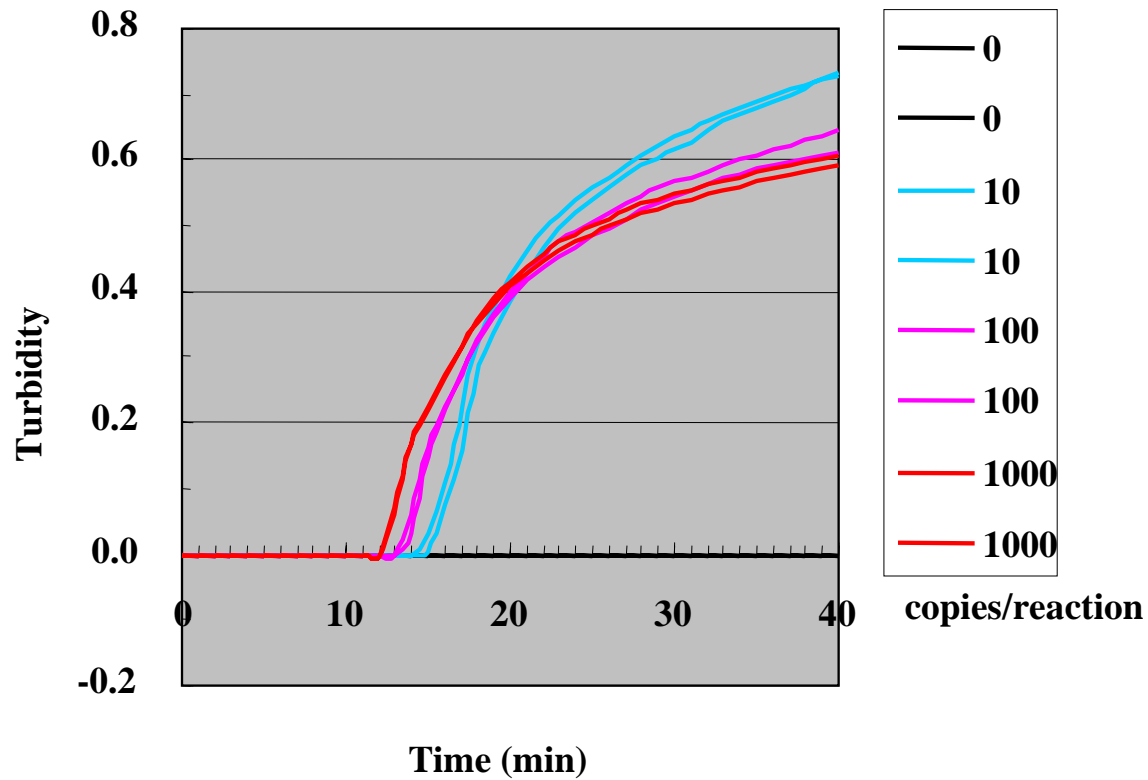
Development of LAMP assay for detection of Mycobacterium tuberculosis complex (TB-LAMP)

Analytical specificity of TB-LAMP



Template: purified genomic DNA (10⁶ copies/ reaction)

Analytical sensitivity of TB-LAMP



Template: purified genomic DNA

Whole Procedure of TB-LAMP

Sample preparation

Decontamination of sputum sample with NALC-NaOH



Boiling of the sample to lyse bacteria



Purification of DNA using Extraction device



Amplification

Detach the DNA-trapped Microweb tip into the reaction mixture containing Calcein reagent



LAMP reaction at 65C for 40min



Detection

Detection of visible fluorescent signal

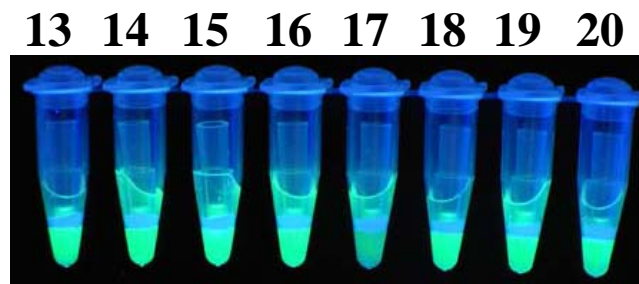
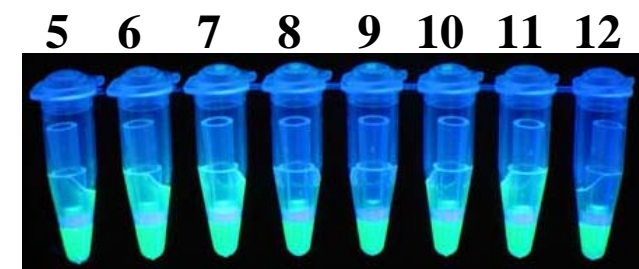
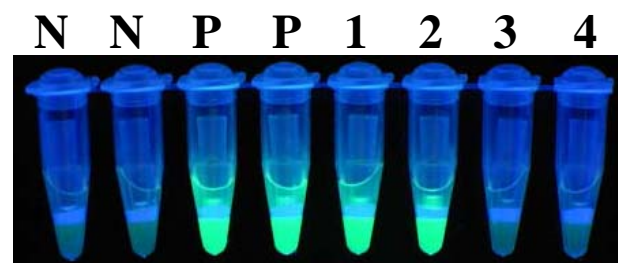
Extraction device



Microweb tip

TB-positive specimens

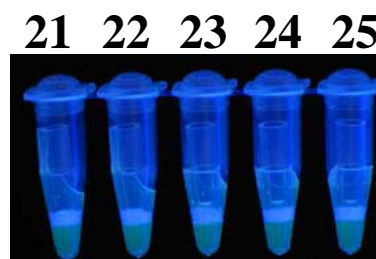
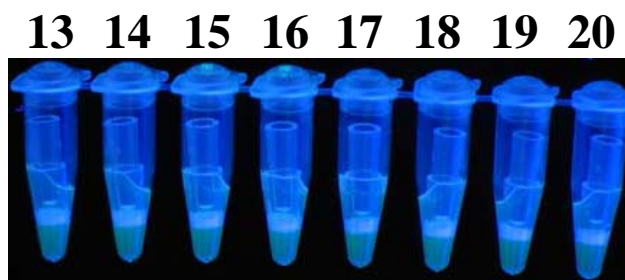
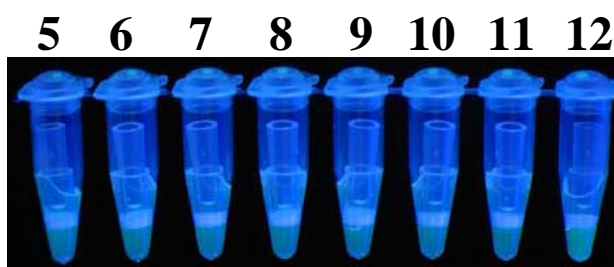
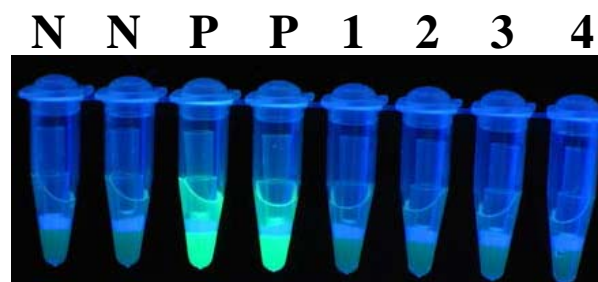
Specimen No.	Smear result	Amplicor result	LAMP result
1	-	TB	+
2	-	TB	+
3	G1	TB	- *
4	-	TB	- *
5	-	TB	+
6	G3	TB	+
7	G8	TB	+
8	-	TB	+
9	-	TB	+
10	G1	TB	+
11	G1	TB	+
12	G1	TB	+
13	-	TB	+
14	G1	TB	+
15	G1	TB	+
16	G2	TB	+
17	-	TB	+
18	G2	TB	+
19	G1	TB	+
20	G5	TB	+
NC			-
PC			+



(No.3*,4*:Sputum samples in small volumes were used)

TB-negative specimens

Specimen No.	Smear result	Amplicor result	LAMP result
1	G3	<i>M. avium</i>	-
2	-	<i>M. avium</i>	-
3	-	<i>M. avium</i>	-
4	-	<i>M. avium</i>	-
5	G1	<i>M. avium</i>	-
6	-	<i>M. avium</i>	-
7	-	<i>M. avium</i>	-
8	G1	<i>M. intraceluurae</i>	-
9	-	<i>M. intraceluurae</i>	-
10	G6	<i>M. intraceluurae</i>	-
11	-	-	-
12	-	-	-
13	-	-	-
14	-	-	-
15	-	-	-
16	-	-	-
17	-	-	-
18	-	-	-
19	-	-	-
20	-	-	-
21	-	-	-
22	-	-	-
23	-	-	-
24	-	-	-
25	-	-	-
NC			-
PC			+



Summary of Preliminary Data of TB-LAMP

		TB-LAMP		
		+	-	
Amplicor	+	18	2	20
	-	0	25	25
		18	27	

- Results of TB-LAMP showed good correlation with the Amplicor's results.
- Concordance rate of TB-LAMP indicating TB-positive was 90% (18/20; with Amplicore positive samples).
- Results of TB-LAMP gave good agreement with the results of Amplicor (25/25; in case of smear negative samples).

Sample ID	Culture	AFB	NALC-NaOH		Direct method		Comments
		(score)	Turbidity Tt (min)	Fluorescent visual detection	Turbidity Tt (min)	Fluorescent visual detection	
100364	-	0	N.D./-	-	N.D./-	-	
100367	-	0	N.D./-	-	N.D./-	±	
100374	-	0	N.D./-	-	N.D./-	-	
100375	-	0	N.D./-	-	N.D./-	-	
100376	-	0	N.D./-	-	N.D./-	-	
100377	-	0	N.D./-	-	N.D./-	-	
100379	-	0	N.D./-	-	N.D./-	-	
100380	-	0	N.D./-	±	N.D./-	-	
100384	-	0	N.D./-	-	N.D./-	±	
100385	-	0	N.D./-	-	N.D./-	±	
200252	+	1+	19.4/+	+	14.7/+	+	
200260	+	1+	17.6/+	+	18.4/+	+	
200262	+	1+	23.6/+	+	16.4/+	+	
200278	+	1+	25.3/+	+	17.6/+	+	
200286	+	1+	26.7/+	+	21.3/+	+	
200300	+	1+	21.8/+	+	15.2/+	+	
200323	+	1+	19.9/+	+	20.5/+	+	
200336	+	1+	24.7/+	+	15.8/+	+	
200339	+	1+	N.D./-	-	N.D./-	-	not homogenous
200341	+	1+	30.5/+	+	N.D./-	-	not homogenous

Sample ID	Culture	AFB	NALC-NaOH		Direct method		Comments
		(score)	Turbidity Tt (min)	Fluorescent visual detection	Turbidity Tt (min)	Fluorescent visual detection	
200250	+	2+	22.1/+	+	15.9/+	+	
200259	+	2+	20.1/+	+	14.4/+	+	
200275	+	2+	24.2/+	+	15.6/+	+	
200277	+	2+	20.0/+	+	16.2/+	+	
200296	+	2+	20.9/+	+	15.0/+	+	
200301	+	2+	20.1/+	+	16.3/+	+	
200305	+	2+	18.9/+	+	15.8/+	+	
200306	+	2+	20.7/+	+	15.7/+	+	
200311	+	2+	18.7/+	+	16.5/+	+	
200322	+	2+	19.5/+	+	19.1/+	+	
200324	+	2+	15.1/+	+	13.3/+	+	
200333	+	2+	13.9/+	+	15.1/+	+	
200337	+	2+	17.5/+	+	14.8/+	+	
200338	+	2+	18.2/+	+	16.9/+	+	
200063	+	3+	18.2/+	+	18.2/+	+	
200270	+	3+	17.4/+	+	15.3/+	+	
200271	+	3+	14.9/+	+	16.2/+	+	
200273	+	3+	17.7/+	+	15.4/+	+	
200280	+	3+	14.7/+	+	14.8/+	+	
200285	+	3+	16.7/+	+	15.3/+	+	
200287	+	3+	15.3/+	+	15.1/+	+	
200288	+	3+	18.3/+	+	19.6/+	±	bloody sputum
200290	+	3+	16.5/+	+	13.8/+	+	
200291	+	3+	14.3/+	+	14.2/+	+	
200293	+	3+	15.3/+	+	13.9/+	+	
200297	+	3+	18.5/+	+	13.8/+	+	
200298	+	3+	15.7/+	+	14.4/+	+	
200299	+	3+	N.D./-	-	N.D./-	-	not homogenous

Evaluation of Direct method with sputum specimens

	NALC-NaOH			Direct method			
	+	+/-	-	+	+/-	-	
Culture -	0	1	9	0	3	7	10
Culture +	36	0	2	34	1	3	38
	36	1	11	34	4	10	

- a) In general, Direct method showed good correlation with the NALC method.
- b) There were a few samples, which showed discrepant results which could have been due to the following reasons.
 - interference with the fluorescence signal by Haeme
 - non-homogeneous samples and insufficient samples
 - false labeling of the samples
- c) Eiken shall investigate further samples.

Conclusion

TB-LAMP assay provides:

- **Rapid:**

Results can be obtained within two hours including DNA extraction.

- **Simple:**

Whole assay procedure is very simple and easy to operate. It does not require any sophisticated equipments.

- **Specific:**

It detects TB complex specifically (no cross reaction with NTB).

- **Sensitive:**

10 copies of TB DNA can be detected.

EIKEN will contribute to the global health through LAMP based diagnostic tools.



Acknowledgment: We would like to express great appreciation to FIND for providing us precious samples and valuable advise.