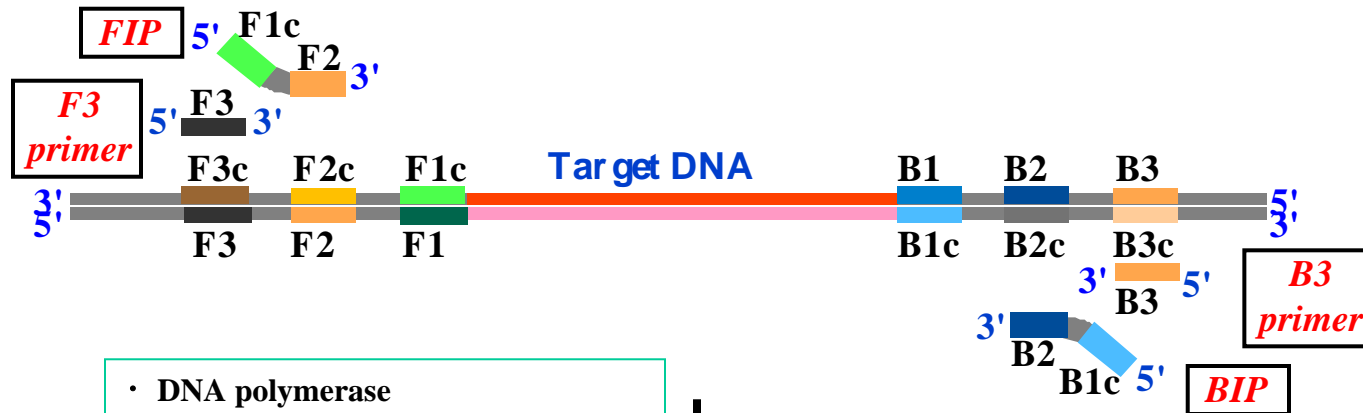


# Progress on the LAMP assay for TB diagnosis



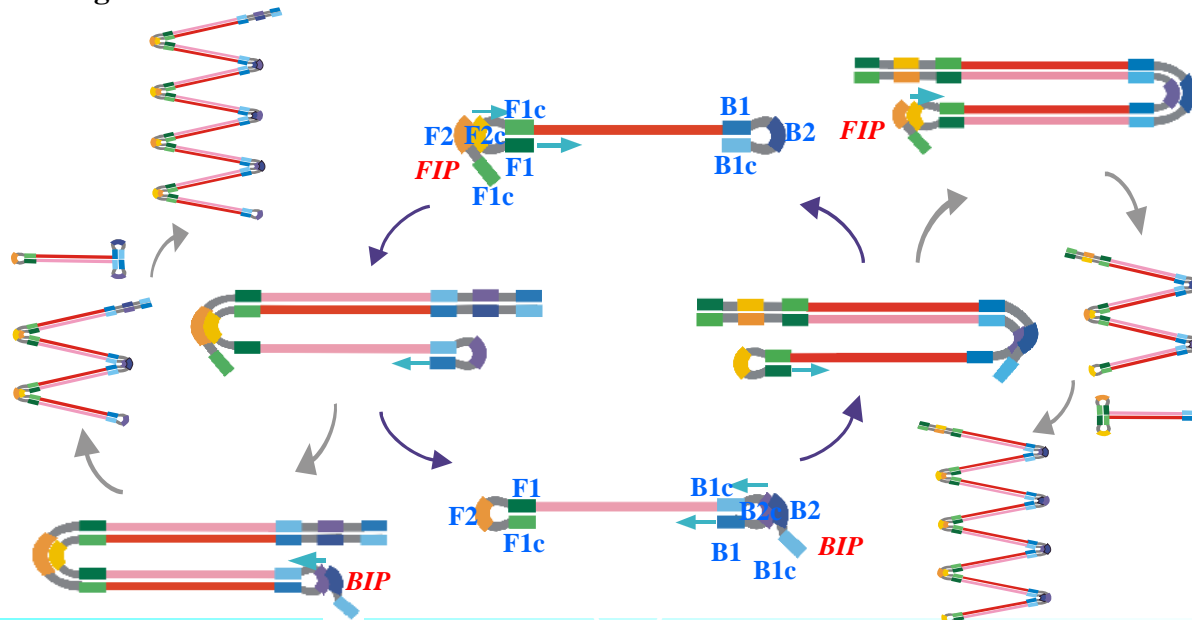
**Tetsu Hase, Hidetoshi Kanda, Tsugunori Notomi  
and Junji Morikawa (Eiken Chemical Co., Ltd.)**

# Mechanism of LAMP reaction

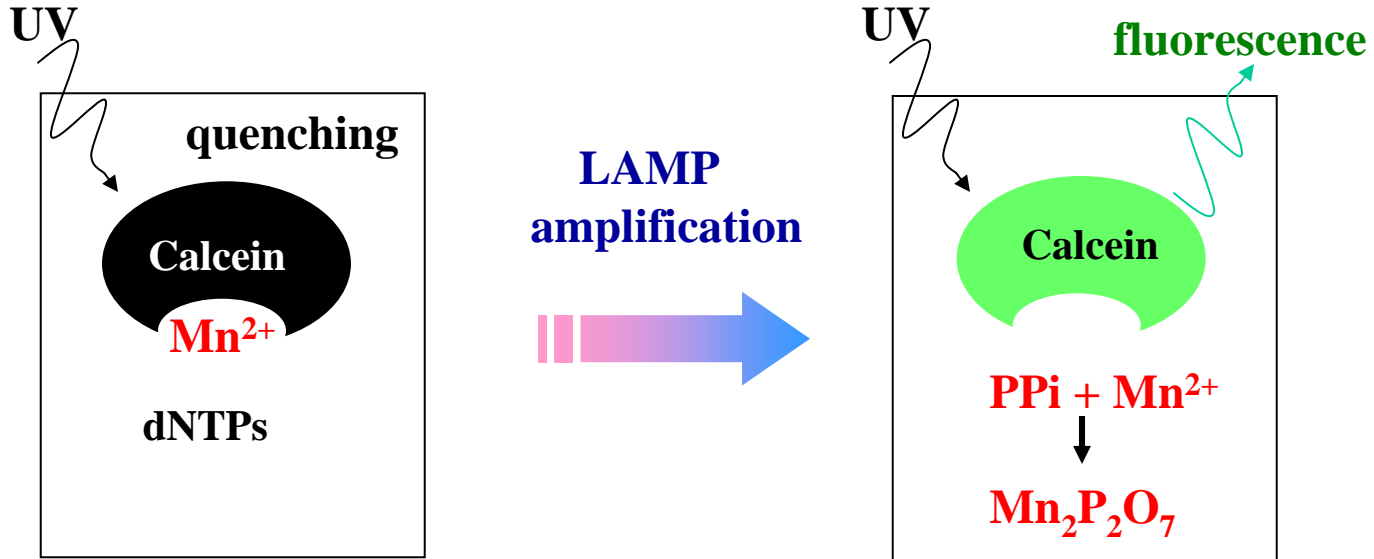


- DNA polymerase (with strand displacement activity)
- dNTPs
- $Mg^{++}$

Incubate at 60°C ~ 65°C



# Detection - visible fluorescence -



- \* No need of special equipment for the detection (only UV lamp is required).
- \* It enables to avoid cross contamination since it is performed in a closed tube from amplification to detection.



- + (TB)

# Procedure of LAMP method

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



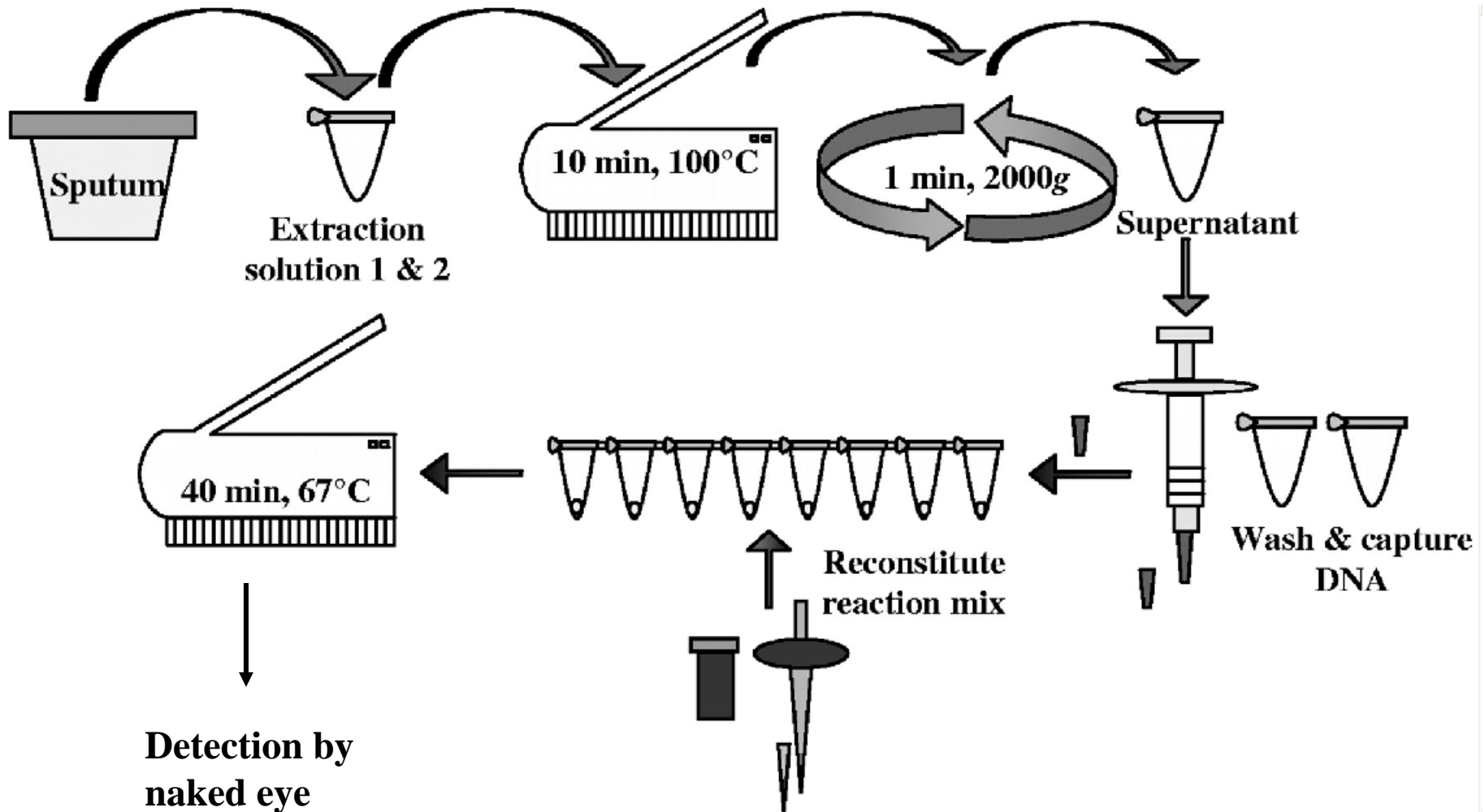
**60 – 65C**  
**15 - 60 min.**

**Detection**

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

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

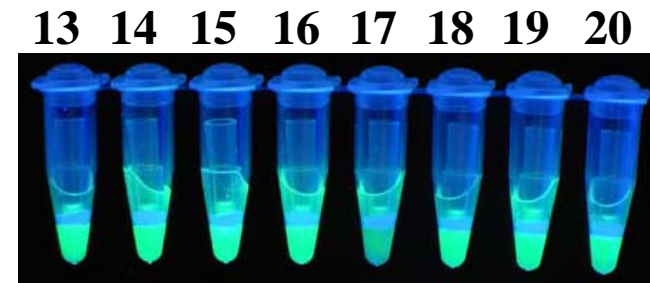
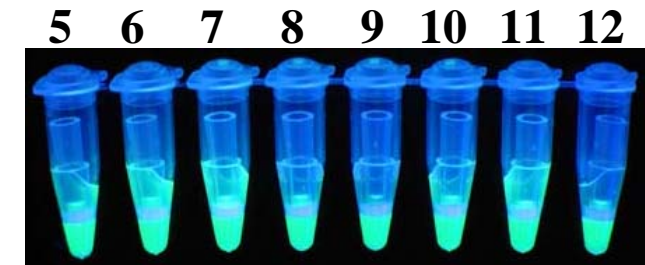
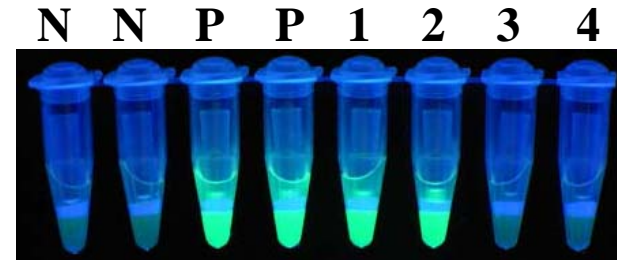
# TB-LAMP Assay



(C. Boehme *et. al.*, J. Clin. Microbiol. 2007 45: 1936-1940. )

# TB-positive specimens

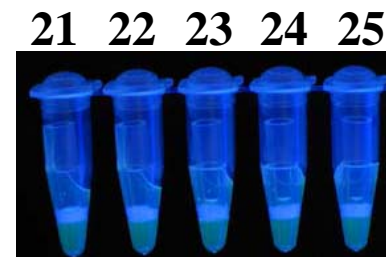
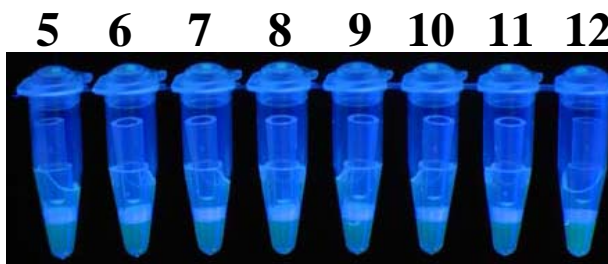
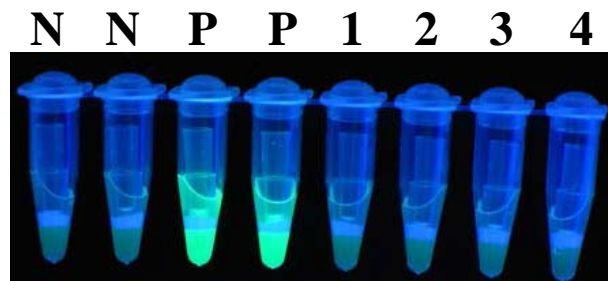
| Specimen No. | Smear result | Amplicor result | LAMP result |
|--------------|--------------|-----------------|-------------|
| 1            | -            | TB              | +           |
| 2            | -            | TB              | +           |
| 3            | G1           | TB              | - *         |
| 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           |              |                 | +           |



\*; Small specimen volume (50%)

# TB-negative specimens

| Specimen No. | Smear result | Amplificor 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           |              |                         | +           |

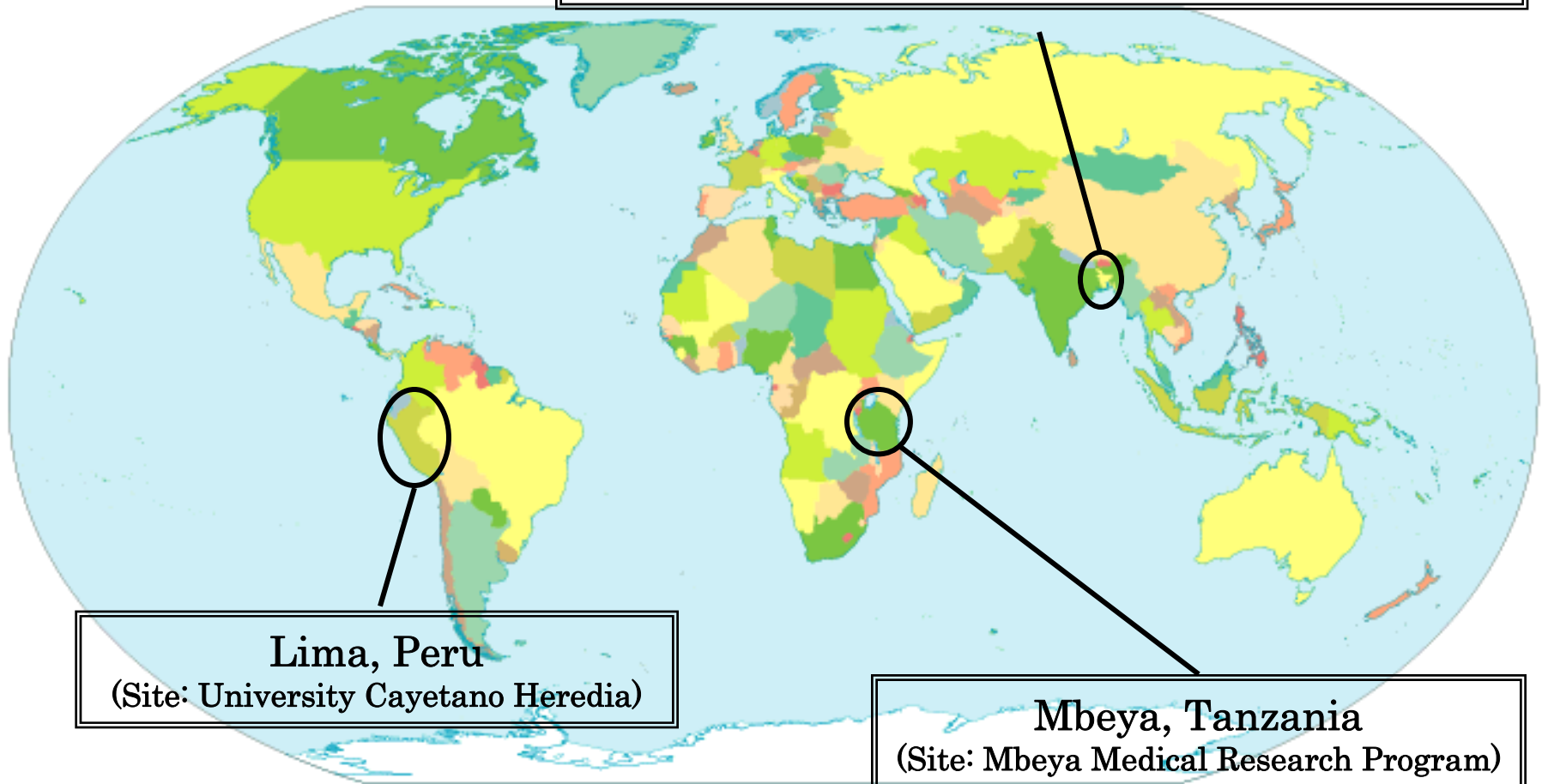




# TB-LAMP Feasibility Study

(Conducted by FIND-EIKEN; Feb. – May, 2006)

**Dhaka, Bangladesh**  
(Site: ICDDR [International Centre for Diarrhoeal Disease Research])



**Lima, Peru**  
(Site: University Cayetano Heredia)

**Mbeya, Tanzania**  
(Site: Mbeya Medical Research Program)

## Results of the TB-LAMP Feasibility Study

### **1) Clinical performance**

**a) Sensitivity (in smear +, LJ +) : 95.5-99.9% (av. 97.7%; 173/177)**

**b) Sensitivity (in smear -, LJ +): 32.0-63.0% (av. 47.5%; 19/40)**

**c) Specificity (in smear -, LJ -): 98.1-99.9% (av. 99.0%; 509/514)**

**2) The hands-on-time for the testing was similar to that of sputum smear microscopy.**

**3) No indeterminate results were reported and the inter-reader variability was 0.4%.**

**4) Despite the use of a simple room for all procedures, no DNA contamination was observed.**

**5) The assay was robust with high end-point stability and low rate of test failure.**

**6) Technologists with no prior molecular experience were able to easily perform the assay.**

## Current TB-LAMP assay is:

### <Usability>

- **Rapid:**

- \* Results can be obtained within 100 min. including the DNA extraction.

- **Easy-to-use:**

- \* Entire procedure is very simple and easy to operate.

- \* It does not require any sophisticated equipment.

- \* However simpler assay with less steps is desirable.

### < Performance >

- **Specific:**

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

- **Sensitive:**

- \* Detection rate of smear positive sample is high.

- \* Higher sensitivity is required for the detection of smear negative, culture positive sample.

Now we are focusing on the improvement of TB-LAMP assay to be

**more sensitive with a simpler operation.**

# Acknowledgments

## *FIND*

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**Dr. M. Perkins**

**Dr. C. Boehme**

## *Kobe Institute of Health, Japan*

**Dr. M. Ito**

**Dr. T. Iwamoto**

## *Feasibility study sites*

**\* Dr. M. Hoelscher (*U. Munich, Germany*)**

**\* Dr. P. Nabeta , Dr. G. Henostroza**

**and Dr. E. Gotuzzo**

**(*U. Peruana ayetano Heredia, Peru*)**

**\* Dr. R. Raqid (*ICDDR, Bangladesh*)**



**EIKEN will continue to contribute to the health of people  
as a general manufacturer of clinical diagnostics.**

**Thank you for your kind attention.**