Original Article

# Comparative study of the loop-mediated isothermal amplification method and the QIAGEN *therascreen* PCR kit for the detection of EGFR mutations in non-small cell lung cancer

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**Background:** Epidermal growth factor receptor (*EGFR*) mutations are important biomarkers in the treatment of patients with advanced or metastatic diseases. The *therascreen* EGFR Rotor-Gene Q (RGQ) PCR Kit® (Qiagen, Inc.) is an approved diagnostic test for EGFR mutations in non-small cell lung cancer (NSCLC). This study aims to investigate the diagnostic capability of a loop-mediated isothermal amplification (LAMP) assay as an accurate, efficient, and cost-effective alternative to the *therascreen* assay.

**Methods:** EGFR mutations were investigated by LAMP and *therascreen* assays using tissue samples that were surgically resected or biopsied from 117 consecutive patients with NSCLC tumors. The EGFR status from the LAMP assay was compared with that of the *therascreen assay*. Next-generation sequencing (NGS) was performed to confirm EGFR status of tumors that did not match in both assays. To establish an optimal LAMP AUC value, receiver operating characteristics (ROC) curve analysis was performed within tumors with exon 19 deletion or L858R point mutation.

**Results:** Of the 117 tumors assayed, 45 tumors with *EGFR* mutations and 68 tumors with *EGFR* wild type were matched in both assays, four tumors having mismatched EGFR statuses. NGS further confirmed that two of the four discordant tumors had the same EGFR status that was determined by the LAMP assay. The AUC values were 0.973 (95% CI: 0.929–1.00) in exon 19 deletion, and 0.952 (95% CI: 0.885–1.00) in L858R point mutation. In exon 19 deletion, sensitivity, specificity, and accuracy were 89.3%, 98.9%, and 96.6%, respectively, and 94.7%, 95.9%, and 95.7%, respectively, in L858R using AUC value of 0.222.

**Conclusions:** The LAMP assay compared favorably with the *therascreen* assay and has potential as an effective, simple, rapid, and low-cost diagnostic alternative. Based on these results, a liquid biopsy LAMP system should be developed for point-of-care testing of oncogenes in the near future.

**Keywords:** Lung cancer; adenocarcinoma; epidermal growth factor receptor (*EGFR*); loop-mediated isothermal amplification (LAMP); polymerase chain reaction

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## Introduction

Lung cancer, accounting for 2.09 million new cases and 1.76 million deaths in the GLOBOCAN 2018 database (1) and 2.28 hundred thousand new cases and 1.42 hundred thousand deaths in the United States (2), is the most common cancer and the leading cause of death from cancer in many developed countries. However, overall survival and progression-free survival have improved for patients with advanced non-small cell lung cancer (NSCLC) who have specific predictive biomarkers and have received molecular targeted therapy or immunotherapy compared with those receiving cytotoxic chemotherapy (3-10). Currently, almost all guidelines for lung cancer strongly recommend the investigation of several established biomarkers (*EGFR* mutations, *ALK* and *ROS1* rearrangement, *BRAF V600E* point mutations, and PD-L1 expression levels) prior to initial treatment of patients with advanced NSCLC (11-16).

NSCLC with *EGFR* mutations is highly responsive to EGFR tyrosine kinase inhibitors (TKIs), such as gefitinib (Iressa®; AstraZeneca UK Limited) (17). Gefitinib was approved for the first-line treatment of patients with metastatic NSCLC on July 5, 2002 in Japan (18), and was later approved for metastatic NSCLC patients on May 5, 2003 in the United States. Simultaneously, the therascreen EGFR Rotor-Gene Q (RGQ) PCR Kit® (*therascreen* assay; Qiagen, Inc.) was approved as a companion diagnostic test by the Ministry of Health, Labour and Welfare in Japan and by the United States Food and Drug Administration (FDA) (19,20).

The *therascreen* EGFR RGQ PCR Kit® is a commercial real-time polymerase chain reaction (RT-PCR) test kit, which detects 21 *EGFR* mutations in exons 18, 19, 20, and 21 against a background of wild type genomic DNA. After purification of DNA extracted from formalin-fixed paraffin-embedded (FFPE) tissue, aberrant DNA is amplified using amplification refractory mutation system (ARMS) primers with Rotor-Gene Q MDx® (Qiagen, Inc., Tokyo, Japan), which is a dedicated thermal cycler. While this assay can be performed within eight hours, it requires the use of sophisticated equipment and procedures, and specimens that are adequate sample size and of a high quality.

Recently, next-generation sequencing (NGS), which enables massively paralleled DNA sequencing, has been introduced into practical clinics in some developed countries (21-25). Since this technology is able to sequence millions of target genes simultaneously, many oncologists are expecting that the genotyping of lung cancer will be more cost- and time-effective. However, NGS has several limitations: (I) expensive equipment; (II) requires experienced technicians; (III) requires experienced biostatisticians; and (IV) it is time-consuming. NGS is particularly unsuited for patients with far advanced disease because EGFR status should be investigated as soon as possible to allow fast precision medicine and first-line therapy with EGFR-TKIs.

Loop-mediated isothermal amplification (LAMP) is an alternative PCR based technology with high levels of specificity and amplification efficiency under isothermal conditions (26-28). It is being used worldwide in bacteriology (29-32), protozoology (33), mycology (34), and virology including SARS-CoV-2 (35-41). This assay has the following advantages: (I) high speed detection within 30 minutes after the amplification reaction begins; (II) unpurified samples can be used directly; and (III) high sensitivity and specificity compared with conventional PCR methods. Furthermore, the cost-effectiveness of LAMP was proved in tuberculosis by World Health Organization because no expensive thermal cycler is needed (42). Although the LAMP assay has not be put into practice used in clinical oncology, it could provide more rapidly, simply, and inexpensively method for detecting oncogene.

In this study, we aimed to develop a new point-of-care testing LAMP assay for detecting EGFR mutations in oncology. To evaluate the sensitivity and specificity, we compared results between the LAMP assay and the *therascreen* assay. Additionally, by analyzing the area under the receiver operator characteristic curve (ROC), we calculated the optimal threshold that distinguishes between EGFR mutations (exon 19 deletion or L858R) and the wild type. We present the following article in accordance with the STARD reporting checklist (available at http://dx.doi.org/10.21037/jtd-20-2642).

## Methods

### *Study design*

This study was a prospective study, without intervention, that was designed to elucidate the sensitivity and specificity of the LAMP assay compared with the *therascreen* assay. Clinical data and specimens were collected from consecutive resected tumor tissues from patients at Saitama Cardiovascular and Respiratory Center (Saitama, Japan). The present study was approved by The Institutional Review Board of the Saitama Cardiovascular and Respiratory Center (approval No. 2018032 and 2019038).

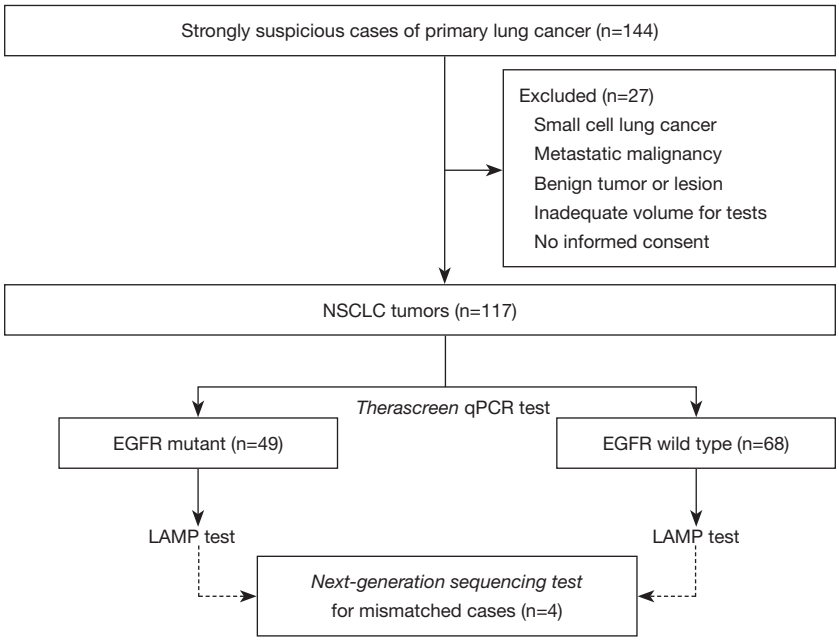


**Figure 1** Flowchart of registration. Candidate cases were strongly suspicious primary lung cancer preoperatively. After surgery, all cases without primary non-small cell lung cancer and/or informed consent were excluded from the study. Among candidate cases (n=144), 117 samples were available for testing both *therascreen* and LAMP assays. Finally, next-generation sequencing was performed for the purpose of investigating *EGFR* status of discordant cases between *therascreen* and LAMP assays. NSCLC, non-small cell lung cancer.

Written informed consent was provided by all patients. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). There were two primary end points for the study: (I) reproducibility of sensitivity and specificity by using another larger cohort than our previous series, and (II) LAMP AUC value by ROC analysis. All patients with suspicious primary lung cancer underwent surgery or surgical biopsy. After excluding cases of no malignancy or pulmonary metastasis from any other cancer, each specimen was examined microscopically to determine whether an adequate amount of tumor cells was present.

### *Study population*

We aimed to collect 50 *EGFR* mutated tumors and over 60 *EGFR* wild type tumors, since at least one tumor with mismatched EGFR status could be expected between the *therascreen* and LAMP assays, based on results in our preliminary study.

### *Tumor tissue samples*

Total 144 samples were obtained for this study from patients with a strong suspicion of primary lung cancer at The Saitama Cardiovascular and Respiratory Center between January 2019 and January 2020 (*Figure 1*). Among of them, 117 tumor tissues were considered eligible from participants in this study. The mean age of participants was 69.1 years, and they consisted of 77 males and 40 females. Before surgery or biopsy, written informed consent was obtained from consecutive patients with a strong suspicion of primary lung cancer. All specimens were diagnosed by an expert pulmonary pathologist examining HE-stained slides from a low magnification to a high magnification under an ECLIPSE Ni-u light microscope (Nikon Co., Ltd., Tokyo, Japan). Diagnoses were based on the WHO classification version 8. The inclusion criteria were as follows: (I) NSCLC; (II) enough material volume for molecular testing; and (III) informed written consent from each patient. Cases with small cell lung cancer, pulmonary metastasis from other cancers, absence of informed consent, or insufficient sample volume were excluded. All specimens were fixed with 10% buffer formalin at 20–25 ℃ (24–36 h) to create formalin-fixed, paraffin-embedded (FFPE) tumor blocks according to the recommendation of The Japanese Society of Pathology (43). Hematoxylin-eosin staining

was performed by the standard methods using Tissue-Tek Prisma® (Sakura Finetek Japan Co., Ltd., Tokyo, Japan) according to the manufacturer's protocol.

### *DNA extraction*

To ensure efficient PCR amplification, the tumor content of each sample was assessed by light microscopy at magnifications ×10 to ×100. After sections were deparaffinized with xylene and hydrated through a graded series of ethanol (100%, 100%, 85%, and 70% ethanol), DNA from the tissue blocks was extracted using the QIAampTM DNA FFPE Tissue kit® (Qiagen, Inc., Tokyo, Japan) and analyzed using a QIAcube Robot® (Qiagen, Inc., Tokyo, Japan) according to the manufacturer's protocols (44).

### *Therascreen qPCR mutation analysis*

The presence of EGFR mutations was determined using a therascreen EGFR PCR kit® (Qiagen, Inc., Tokyo, Japan) according to the manufacturer's protocols (45).

### *LAMP mutation analysis*

A primer set for targeting EGFR mutation genes was designed using Primer Explorer (primerexplorer.jp/e/) and synthesized by Eurofin Genomics (Eurofins Genomics K.K., Tokyo, Japan). Block oligo and fluorophore-labelled probes were synthesized and purified by Japan Bio Services (Japan Bio Service Co., Ltd., Saitama, Japan) or Gene Design (Gene Design Inc., Osaka, Japan) The LAMP assay was conducted at 65 ℃ for 120 minutes by LightCycler 480® (Roche Diagnostics K.K.), as described in our previous study (46). After denaturing the amplicon at 95 ℃ for 5 min followed by hybridization at 37 ℃ for 5 min, the temperature was gradually raised to 80 ℃ and the fluorescent intensity was measured 7 times per 1 ℃ increment. The resulting data were analyzed by LightCycler 480 software® (version 1.5.1.62; Roche Diagnostics K.K., Tokyo, Japan) to calculate melting peak.

### *NGS*

NGS was performed for discordant *EGFR* status cases between *therascreen* and LAMP assays. Amplicon-based NGS was performed using MiSeq system (Illumina K.K., Tokyo, Japan). The primer sets for amplification of EGFR exons 18, 19, 20, and 21 were used as previously reported (46). The resulting FASTQ files were mapped to GRCh38 human reference sequence by using BWA-MEM (http://bio-bwa.sourceforge.net/). The variant data was extracted from the mapped data by Samtools ver. 1.9 (http://www.htslib.org/) and GATK4 (https://gatk.broadinstitute.org/hc/en-us).

### *Statistical analysis*

Descriptive statistics, categorical variables, and the sensitivity and specificity of the LAMP assay were calculated using standard formulae with Excel 2019 ver. 16.0.12527.20260® (Microsoft Corp., Tokyo, Japan) and SPSS Statistics® version 24 (IBM Corp., Armonk, NY, USA). Standardization of LAMP values and ROC analyses were performed using the statistical software R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria) with ROCR package. Clinical and pathological findings were extracted from the electronic medical records of the patients. To minimize the scattering of LAMP values among primers in the respective locus of *EGFR* mutations (for example, 18 kinds of primers were used in the LAMP assay to investigate exon 19 deletion), the standardization was performed mathematically to calculate and generate a beeswarm plot of LAMP values. ROC curve analysis was constructed to determine maximum sensitivity and specificity. Under the hypothesis that the EGFR status in the *therascreen* method would be true, the ROC curve depicted the relationship between true-positive (sensitivity) and false-positive (1 – specificity) cases. The accuracy of the LAMP test was presented by the area under the curve (AUC) and a parametric method based on bivariate normal distribution was used to implement a maximum likelihood estimator for fitting a smooth curve to the data points. The diagnostic AUC values for tumors with and without *EGFR* mutation were chosen as those values that corresponded to the points on the ROC curves, nearest the upper left hand corner of the graph for optimal balance between sensitivity and specificity. $P<0.05$ was considered significant.

## Results

### *Characteristics of patients with NSCLC*

The mean age of the 117 patients was 69.1 years (35–85 years) (*Table 1*). The majority of patients were male (n=77, 65.8%), while 40 patients were female (34.2%). In terms of smoking habit, there were 32 never-smokers

**Table 1** Clinical characteristics of study patients

| Characteristic | N (%) |
|---|---|
| Age, years | 69.1±9.6 |
| Gender | |
| Male | 77 (65.8) |
| Female | 40 (34.2) |
| Smoking Status | |
| Never smoker | 32 (27.4) |
| Former smoker | 74 (63.2) |
| Current smoker | 10 (8.5) |
| Histology | |
| Adenocarcinoma | 92 (78.6) |
| Squamous cell carcinoma | 20 (17.1) |
| Adenosquamous cell carcinoma | 3 (2.6) |
| Pleomorphic carcinoma | 1 (0.9) |
| Large cell neuroendocrine carcinoma | 1 (0.9) |
| Pathological stage | |
| P0 | 1 (0.9) |
| pIA1 | 22 (18.8) |
| pIA2 | 35 (29.9) |
| pIA3 | 13 (11.1) |
| pIB | 17 (14.5) |
| pIIA | 6 (5.1) |
| pIIB | 9 (7.7) |
| pIIIA | 7 (6.0) |
| pIIIB | 1 (0.9) |
| pIIIC | 1 (0.9) |
| pIVA | 3 (2.6) |
| pIVB | 2 (1.7) |

Data on age: mean ± SD.

(27.4%), 74 former-smokers (63.2%), and 10 current smokers (8.5%). Ninety-two cases (78.6%) were diagnosed with adenocarcinoma, 20 (17.1%) with squamous cell carcinoma, 3 (2.6%) with adenosquamous cell carcinoma, 1 (0.9%) with pleomorphic carcinoma, and 1 (0.9%) with large cell neuroendocrine carcinoma. The number of patients in pathological stage 0, IA1, IA2, IA3, IB, IIA, IIB, IIIA, IIIB, IIIC, IVA, IVB was 1 (0.9%), 22 (18.8%), 35 (29.9%), 13 (11.1%), 17 (14.5%), 6 (5.1%), 9 (7.7%), 7 (6.0%), 1 (0.9%), 1 (0.9%), 3 (2.6%), and 2 (1.7%), respectively.

**Table 2** Association between the results from the LAMP assay and the *therascreen* assay

| EGFR status | *Therascreen* PCR | |
|---|---|---|
| | Positive | Negative |
| LAMP | | |
| Positive | 47 | 2 |
| Negative | 2 | 66 |

EGFR, epidermal growth factor receptor; LAMP, loop-mediated isothermal amplification.

### *Therascreen EGFR PCR mutation analysis*

Among the 117 NSCLCs, there were 49 tumors with *EGFR* mutations (*Table 2*), including 25 tumors of exon 19 deletion, 19 tumors of exon 21 L858R point mutations, 2 tumors of exon 19 deletion/exon 20 in-frame insertion (double mutation), 1 tumor of exon 20 in-frame insertion, and 2 uncommon *EGFR* mutations (exon 18 G719X point mutation, and double mutation of exon 19 deletion/exon 18 G719X point mutation) (*Table 3*).

### *LAMP EGFR mutation analysis*

The LAMP assay detected 49 *EGFR* mutations (*Table 2*), however, four mismatched cases were included in *therascreen* assay. Among the 49 *EGFR* mutations, there were 26 tumors of exon 19 deletion, 21 tumors of exon 21 L858R point mutations, and 2 uncommon *EGFR* mutations (exon 18 G719X point mutation, and double mutation of exon 21 L858R point mutation/exon 18 G719S point mutation) (*Table 3*).

### *Comparison of results of therascreen PCR and LAMP assays*

Among the 117 tumors, there were 47 tumors with *EGFR* mutations and 66 tumors with *EGFR* wild type in both the *therascreen* and LAMP assays, i.e., a total of 113 tumors with *EGFR* mutation status in the LAMP assay were matched with those in the *therascreen* assay (*Table 2*). The four tumors that did not match were comprised of two tumors with *EGFR* mutation in the *therascreen* assay that were not detected in the LAMP assay, and two tumors with *EGFR*

**Table 3** EGFR mutation statuses identified using *therascreen* or LAMP assays

| *Therascreen* | LAMP | Number of samples |
|---|---|---|
| Del 19 | Del 19 | 24 |
| L858R | L858R | 18 |
| G719X | G719X | 1 |
| Del 19 | L858R | 1 |
| Del 19, G719X | Del 19 | 1 |
| Del 19, Ex 20 Ins | L858R | 1 |
| Del 19, Ex 20 Ins | G719S, L858R | 1 |
| Ex 20 Ins | Negative | 1 |
| L858R | Negative | 1 |
| Negative | Del 19 | 1 |
| Negative | L858R | 1 |
| Negative | Negative | 66 |

EGFR, epidermal growth factor receptor; Exon 18 G719X point mutation, G719X; Exon 19 deletion, Del 19; Exon 20 in-frame insertion, Ex 20 Ins; Exon 21 L858R point mutation, L858R; LAMP, loop-mediated isothermal amplification.

**Table 4** Discrepancy between the results from the LAMP assay and the *therascreen* assay

| Case number | *Therascreen* | LAMP | NGS |
|---|---|---|---|
| Case 1 | L858R | Negative | L858R |
| Case 2 | Ex 20 Ins | Negative | Negative |
| Case 3 | Negative | Del 19 | Del 19 |
| Case 4 | Negative | L858R | Negative |

EGFR, epidermal growth factor receptor; Exon 19 deletion, Del 19; Exon 20 in-frame insertion, Ex 20 Ins; Exon 21 L858R point mutation, L858R; NGS, next-generation sequencing; LAMP, loop-mediated isothermal amplification.

mutation in the LAMP assay that were not detected in the *therascreen* assay. Assuming that the *therascreen* assay can provide a true *EGFR* status of NSCLC tumors, sensitivity and specificity of the LAMP assay were 95.9% and 97.1%, respectively. The positive predictive value and negative predictive value were 95.9% and 97.1%, respectively.

### *NGS for four mismatched cases*

NGS was performed to confirm the *EGFR* mutation disagreement status between the *therascreen* and the LAMP assays. Among the four cases with mismatched EGFR status, NGS demonstrated concordance with the *therascreen* assay in two cases (Case 1 and Case 4) and with the LAMP assay in two cases (Case 2 and Case 3) (*Table 4*).

### *Receiver operating characteristics (ROC) curve analysis of LAMP EGFR mutations*

For the purpose of further improvement of LAMP assay, ROC curve analysis was performed within tumors with exon 19 deletion or L858R point mutation. The area under the ROC curve (AUC) for LAMP values was 0.973 (95% CI: 0.929–1.00) in exon 19 deletion, and 0.952 (95% CI: 0.885–1.00) in L858R (*Figure 2A*,*B*). Maximized sensitivity, specificity, and accuracy were 89.3%, 98.9%, and 96.6%, respectively, based on a cut-off value of 0.806 in exon 19 deletion, and 94.7%, 95.9%, and 95.7%, respectively, based on a cut-off value of 0.222 in L858R.

## Discussion

The identification of *EGFR* mutations has become a standard analysis in the treatment of patients with NSCLC (12-16). However, in spite of the availability of several methods for detecting *EGFR* mutations (47), to date, there are no point-of-care testing methods that offer simplicity, rapid analysis, and are low-cost. More recently, NGS has been rapidly adopted for use in molecular diagnostic examinations to detect aberrant oncogenes in cancers. This method has the advantage of providing multiple hundreds of genomic loci all at once (21-25,48). However, the technology does not currently meet the demands of the remarkable number of samples, especially in Asian countries, that require testing for lung cancer.

*Therascreen* EGFR PCR kit® is a US FDA-approved test for the detection of *EGFR* mutations of lung cancer, and is currently being used in the EU and Japan (19,20). In this study, we compared the performance of the LAMP assay with that of the *therascreen* assay. Among the 117 tumors tested, 113 tumors (96.6%) demonstrated the same EGFR status in each assay, while four tumors differed between the two assays (*Table 2*). The results obtained from the current study were compatible with those from a previous study, which showed sensitivity, specificity, positive predictive value, and negative predictive value of 97.0%, 100%, 100%, and 96.3%, respectively (46).

NGS was performed to investigate the *EGFR* mutations

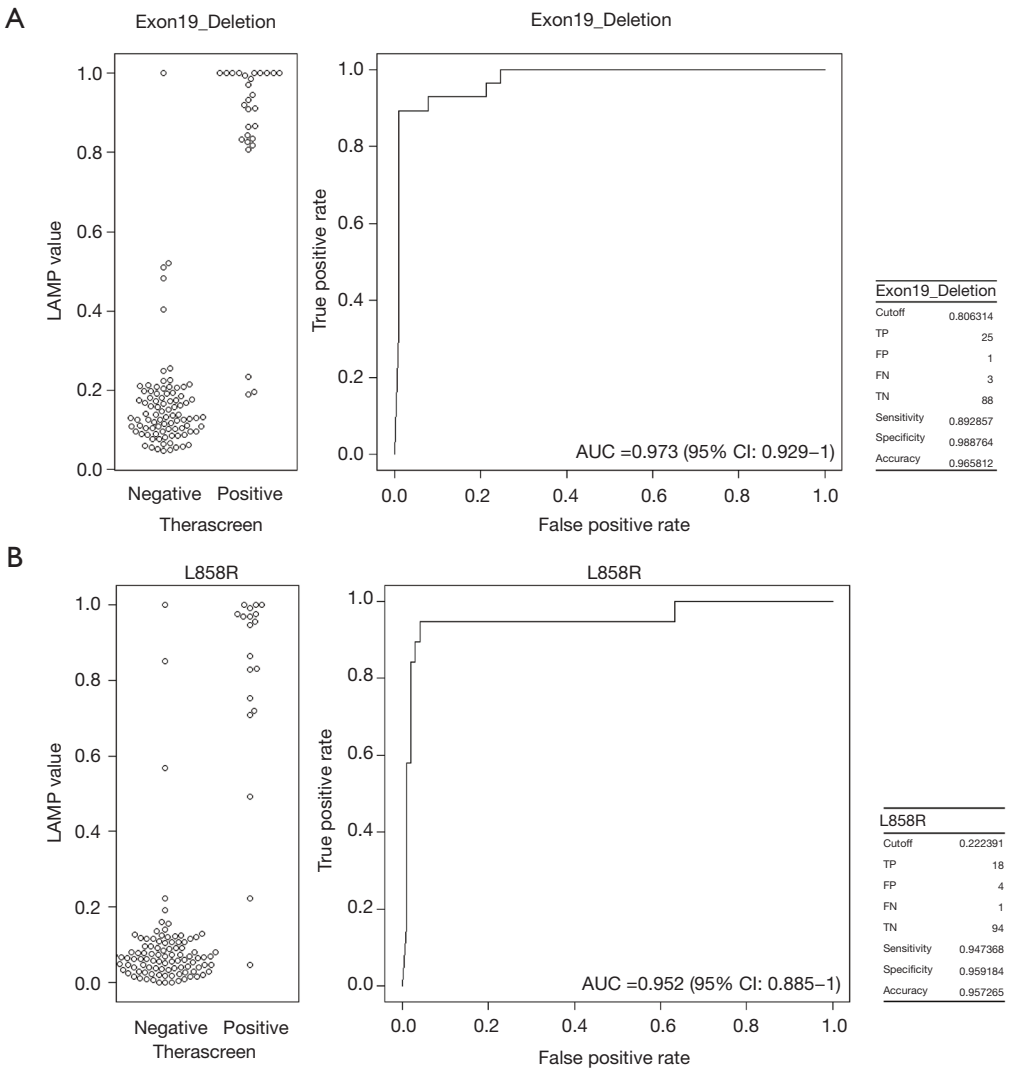


**Figure 2** Results and receiver operating characteristics (ROC) curve of the LAMP assay for EGFR mutations. The ROC curve of exon 19 deletion and L858R are shown in *Figure 2A* and *2B*, respectively. All parameters derived from the ROC curve analysis (cut-off value, sensitivity, specificity, and accuracy) are shown in each figure.

of four tumors that had different EGFR statuses between the two assays. Among the four tumors, NGS EGFR status of half of them was in harmony with LAMP EGFR status. Given these results, a conclusion cannot be drawn as to which assay would be more reliable. Therefore, it is important to understand the limitations in of predicting accuracy of oncogenes in clinical settings, and false positives or false negatives should be taken into consideration even when using tests that are recommended by guidelines.

Among the 117 tumors in this study, 51 tumors demonstrated *EGFR* mutations. In the *therascreen* assay and/or the LAMP assay, five tumors showed "uncommon" *EGFR* mutations. Excluding the uncommon *EGFR* mutations, ROC curves were generated to determine the optimal threshold of LAMP AUC values well distinguish between negative (*EGFR* wild type) and positive (*EGFR* mutations) outcomes of the *therascreen* assay (*Figure 2A*,*B*). The ROC curve of exon 19 deletion gave 0.806 (LAMP value) as a reliable classifier of EGFR status of the *therascreen* assay, for which high specificity (98.9%) and high accuracy (96.6%) were observed, however the sensitivity (89.3%) was moderate (*Figure 2A*). Conversely, all of the

parameters (sensitivity, specificity, and accuracy) were extremely satisfactory in L858R when 0.222 was used as a AUC value (*Figure 2B*). These results could be useful in clinical settings, not only in oncology but also in virology, given the advantages of the LAMP method (rapidity of the test, simplicity of procedure, and versatility of instruments).

In uncommon *EGFR* mutations, five tumors were detected by the *therascreen* assay and/or the LAMP assay in this study (*Table 3*). Among them, only one tumor with G719X was detected by the *therascreen* assay and the LAMP assay (4/5 cases of uncommon *EGFR* mutations were discordant between two assays). Although no prospective large-scale trials have evaluated NSCLC tumors with uncommon mutation, and no standard of treatment has been established to date, the sensitivity to EGFR TKIs may significantly vary. For example, high sensitivity to second-generation EGFR TKIs in exon 18 mutations and S768I exon 20 point mutation, and high sensitivity to osimertinib and poziotinib in exon 20 insertions have been reported (49,50). Therefore, a study of these rare genetic alterations should be conducted in the future and a more accurate detection system for uncommon *EGFR* mutations must be developed.

Currently, the *therascreen* EGFR assay® (Quiagen, Inc.) and the cobas EGFR assay® (Roche Molecular Systems Inc.) for tissue analysis are officially validated by authorized organizations; however, performance of these methods must be improved to meet the global demand in the treatment of various types of EGFR TKIs. Otherwise, effective technological innovations, such as NGS or the LAMP method, must be employed. Despite the limitations of this study (small number of cases and study participants from a single institute), we were able to confirm the feasibility of the LAMP assay for detecting *EGFR* mutations of NSCLC tumors.

This study had several limitations: (I) the relatively small number of cases evaluated; (II) the single institute nature of the study; and (III) no data of cost-effectiveness. The selling price of the EGFR-LAMP assay is not determined at the moment, however, the TB-LAMP assay can be useful reference for the cost-effectiveness of the LAMP assay (42). In the future, multicentre prospective studies may elucidate the comparison between the LAMP assay and other assay.

In conclusion, our study demonstrates that the LAMP assay is a valuable alternative for detecting *EGFR* mutations of NSCLC tumors. This is first report indicating an optimal cut-off value that distinguishes *EGFR* mutations and *EGFR* wild type in the *therascreen* assay. In biology or virology, LAMP assay is already rated highly because of reliability, cost-effectiveness, easy handling, and time shortening of turn-around analysis. Our results show that the LAMP assay could be a suitable candidate for point-of-care testing of *EGFR* mutations in the near future. Now, we are developing a LAMP system for liquid biopsy based on the results from this study (data will be available in our next publication).

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## Footnote

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*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The present study was approved by The Institutional Review Board of the Saitama Cardiovascular and Respiratory Center (approval No. 2018032 and 2019038). Written informed consent was provided by all patients. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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