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A rapid Loop-Mediated Isothermal Amplification (LAMP) test for the detection of somatic variants, p.L858R and p.E746_A750del, in non-small cell lung cancer patients: comparison with real-time PCR and NGS

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ABSTRACT

Background: Identifying tumour-derived somatic variants in the *EGFR* gene is crucial for making treatment decisions in NSCLC patients. The analytical performance and clinical utility of a new LAMP-based portable system for detecting the most frequent somatic variants, p.L858R and p.E746_A750del, using DNA from FFPE samples of NSCLC patients, was evaluated against the reference standards, NGS and real-time PCR.

Methods: This study was non-interventional conducted at the National Center of Oncology in Baku, Azerbaijan following a non-probability purposive sampling, using DNA (n = 44) from FFPE samples. The analytical performance of the LAMP test was evaluated for each somatic variant, p.L858R and p.E746_A750del, using Receiver Operating Characteristic curve analysis. In addition, a questionnaire was developed for healthcare professionals (n = 20) to assess the utility of integrating the new LAMP portable system in clinical practice.

Results: The new LAMP test generated results within 1 h, demonstrating a 95.45 % accuracy in detecting the p.L858R variant compared to real-time PCR and NGS. For the exon 19 deletion, specifically the p.E746_A750del variant, the test achieved 100 % accuracy with NGS. Specificity with real-time PCR was 100 %; however, sensitivity could not be determined, as PCR does not identify which exon 19 deletion was detected. Additionally, the questionnaire revealed unanimous agreement among healthcare professionals and successfully assessed the usefulness of new LAMP portable system, to be integrated as a first diagnostic tool in clinical practice by reducing time, costs and accelerating treatment decisions in NSCLC patients that are positive for the analyzed genetic variants.

Conclusions: The study demonstrates outstanding analytical performance of the new LAMP portable system, highlighting its utility for integration into clinical practice. Particularly beneficial in low-resource settings, this system provides significant value for clinical decision-making in NSCLC patients with positive results, optimizing treatment decisions while saving both time and costs. To our knowledge, this is the first LAMP portable system for oncology applications that can accurately detect somatic variants in the *EGFR* gene.

1. Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide. In 2022, there were 2.48 million new cases, and it remained the leading cause of cancer-related deaths globally, with 1.80 million deaths (Ferlay et al., 2024). Despite advancements in personalised treatments for lung cancer, for non-small cell lung cancer (NSCLC) patients, the global burden of lung cancer disease continues to rise. According with World Health Organization, between 2022 and 2045, the estimated number of new NSCLC cases is projected to increase by 60 %, while

Abbreviations: LAMP, loop-mediated isothermal amplification; NGS, Next Generation Sequencing; NSCLC, non-small cell lung cancer; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; NAAT, nucleic acid amplification test; FFPE, Formalin-Fixed Paraffin-Embedded; SNV, single nucleotide variant; CI, Confidence interval.

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deaths are expected to rise by 70 % worldwide. The situation is even more concerning in low- and middle-income countries (LMICs), where the incidence and mortality rates are anticipated to more than double (International Agency for Research on Cancer, 2024). Personalised treatment decisions for NSCLC patients depend on the analysis of tumour-derived somatic variants, performed in central laboratories using FDA-approved PCR assays, such as the Cobas EGFR and Therascreen EGFR assays, along with NGS cancer panels. While these techniques offer high sensitivity and specificity, their dependence on costly equipment and reagents, the need for specialized training, and the lengthy processing time create significant accessibility barriers—particularly in resource-limited countries. This highlights the urgent need for portable, rapid, and affordable diagnostic solutions, aligning with the global initiative by the Lung Cancer Policy Network (2022), which advocates for the implementation of lung cancer screening programs to enhance accessibility in underserved regions. Loop-Mediated Isothermal Amplification (LAMP) technology is gaining significant attention among rapid diagnostic testing methods. The LAMP market is projected to reach $110.6 million in 2024, with an expected growth to $178.4 million by 2034. Despite the study published 2021 in PharmacoEconomics (Lingervelder et al., 2021) demonstrated that the implementation of LAMP in many countries remains low mainly due to organization of healthcare, the COVID-19 pandemic significantly boosted research based on LAMP technology and it is anticipated that this technology will account for 63.8 % of the market share in clinical diagnostics in 2024 (Future Market Insights, 2024). LAMP is a nucleic acid amplification test (NAAT), that provides a reliable isothermal amplification of DNA in under an hour without the need for thermal cycler, coupled with a high tolerance to inhibitors that often compromise PCR reactions. LAMP technology is mainly being used by major companies like Invitek (“InviScreen® SARS-CoV-2 RT-LAMP”, 2024), New England Biolabs (” SARS-CoV-2 rapid colorimetric LAMP assay kit”, 2024) and Thermo Fisher Scientific (“Colorimetric ReadiLAMP kit for optimized SARS-CoV-2 detection and surveillance using LAMP assay”, 2024) for the detection of SARS-CoV-2 virus. LAMP has also gained attention for the detection of other pathogens, such as, the "Big Three" Infectious Diseases (Makam & Matsa, 2021): Malaria (*Plasmodium* parasite) (Morris & Aydin-Schmidt, 2021), Tuberculosis (*Mycobacterium tuberculosis)* (Jaroenram et al., 2020) and HIV/AIDS (human immunodeficiency viruses) (Hossain et al., 2024).

On the other hand, using the LAMP technology for detecting small genetic variations, such as, single nucleotide variants (SNVs), insertions and deletions, has been challenging, primarily due to the complexity of primer design (Meagher et al., 2018) required to distinguish similar DNA sequences, specially, because the LAMP primers are often tolerant to mismatches (Tamanaha et al., 2022). In addition, small variations in reagent concentrations (Foo et al., 2020; Zhang et al., 2020) and the limited understanding of the biochemical properties of the LAMP enzyme (Oscorbin & Filipenko, 2023) can affect the LAMP reaction efficiency. These parameters become even more critical when LAMP is applied to detect tumour-derived somatic variations, which are often present at low (<10 %) allelic frequencies within a high background of non-mutated DNA sequences. Regarding lung cancer, between 10 % and 20 % of NSCLC patients harbor mutations in the EGFR gene. Deletions in exon 19 and the p.L858R variant in exon 21 together account for approximately 90 % of all cases. These mutations are associated with responsiveness to oral *EGFR* tyrosine kinase inhibitor (TKI) targeted therapy ("Epidermal growth factor receptor (EGFR) mutations in non-small cell lung cancer", 2020). In particular, the p.E746_A750del (also known as E746_A750delELREA) variant (Figs. 1A and 6B) is the most frequent among all exon 19 deletions, although other deletions have also been identified, including p.L747_P753delinsS (Fig. 1B) and p.E746_S752delinsV (Fig. 1C) (Kaneda et al., 2014; Zhao et al., 2020). Most recent data indicate that tumours that do not harbour a sensitizing *EGFR* variant should not be treated with EGFR TKI in any line of therapy (Ettinger et al., 2022).

Few research studies involving lung cancer patients have demonstrated a good performance of the LAMP reaction in detecting EGFR gene variants. However, these studies have been conducted by a single institute using standard thermal cycler equipment (Saito et al., 2021, 2022). However, LAMP technology offers a significant advantage over FDA-approved PCR assays and NGS-based methods due to its compatibility with portable devices. It has the potential to substantially reduce costs and processing time while enabling decentralized diagnostics for optimal patient care, making it particularly valuable in resource-limited settings. A recent comprehensive review underscores the lack of research on LAMP portable devices for oncology applications, particularly in the context of personalised lung cancer treatment. The Doctor Vida® device (STAB VIDA, Caparica, Portugal) is a pocket-sized portable device (dimension, 7 x 4 × 2 cm) using fluorescent real-time LAMP technology. It was initially developed to detect the SARS-CoV-2 virus (Doria et al., 2022) and the single nucleotide variant associated with lactose intolerance (Conceição et al., 2024), achieving an accuracy of over 98 %. Despite the good performance obtained, achieving high sensitivity and specificity for tumour-derived somatic variants present in *EGFR* gene in NSCLC is particularly difficult when attempting to identify low allelic frequency amidst a high background of non-mutated DNA sequences.

The objective of this study was to develop an accurate LAMP-based portable system able to detect the most frequent somatic variants, p.L858R and the exon 19 deletion, p.E746_A750del, in *EGFR* gene and to evaluate its analytical performance at the National Oncology Center in Baku by analyzing the DNA from FFPE samples of NSCLC patients. The performance of the LAMP portable system was then compared against the reference standard techniques, real-time PCR and NGS. In addition, the integration of a portable and affordable LAMP system in clinical practice for treatment decision in NSCLC patients was also evaluated.

Fig. 1. Schematic representation of the DNA sequences of three frequent exon 19 deletions aligned with DNA sequence without mutation (wildtype, WT). NM_005228.5(EGFR) (A) c.2235_2249del (p.E746_A750del), (B) c.2240_2257del (p.L747_P753delinsS), (C) c.2237_2255delinsT (p.E746_S752delinsV).

2. Materials and methods

2.1. Participants and ethical considerations

This research study was approved by the Ethics committee (EKQ/006.23) of the National Center of Oncology in Baku, Azerbaijan. A waiver of informed consent was granted due to the non-interventional nature of the study and the impracticality of obtaining informed consent, as some or all of the participants may have lost follow-up with the institution (they may have moved or passed away). The study was conducted at the National Center of Oncology in Baku, Azerbaijan using DNA from FFPE samples of NSCLC patients which had been previously prepared and stored at the center. Data collection did not interfere with the patients' treatment or referral, and there were no risks to the patients. Patient information was handled confidentially, ensuring full compliance with GDPR regulations and all data were recorded anonymously.

2.2. Sample selection and characterization

This study was conducted following a non-probability purposive sampling with a total of 65 potentially eligible participants from Baku, Azerbaijan. The selection of DNA samples for this study was based on three criteria: (a) a minimum volume of 50 µL, (b) the presence of p.L858R or Exon 19 deletions, and (c) the absence of these variants. DNA quantitation was performed using the Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Out of the 65 potentially eligible participants, only 44 met all criteria and were considered eligible for this study. The same aliquot of the extracted DNA was analyzed by the reference standard techniques (Real-time PCR and NGS) and Index test (LAMP). Fig. 2 illustrates the STARD diagram used in this study with the details on the flow of participants.

2.3. Variant analysis

2.3.1. Real-time PCR

Real-time PCR was performed on DNA samples, following the laboratory routine of Molecular Diagnostic Laboratory at the National Center of Oncology in Baku, Azerbaijan. Briefly, DNA from tissue samples was extracted using the QIAamp DNA FFPE Kit® (Qiagen, Hilden, Germany) and 10 ng of DNA was analyzed with EntroGen's EGFR Mutation Analysis Kit® (EntroGen, Inc., Los Angeles, CA, USA) and CFX96 Touch Real-Time PCR Detection System® (Bio-Rad Laboratories, Hercules, CA, USA) to detect the EGFR variants in exons 18, 19, 20 and 21, following the protocol provided by the test manufacturer.

2.3.2. NGS

Genomic DNA was amplified by PCR using KAPA HiFi HotStart ReadyMix® (Roche Sequencing Solutions, Pleasanton, CA, USA) with the outer specific primers designed for LAMP (F3 and B3), targeting exons 19 and 21 of EGFR gene. 12 ng of generated PCR product were used in the library construction with KAPA HiFi HotStart ReadyMix® (Roche Sequencing Solutions, Pleasanton, CA, USA) and 5 pmol of specific primers with overhang adapters attached (Forward overhang: 5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-[locus specific sequence]-3′ and Reverse overhang: 5′ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-[locus specific sequence]-3'. The generated DNA fragments (DNA libraries) were sequenced with MiSeq Reagent Kit v3 in the MiSeq platform® (Illumina, Inc., San Diego, CA, USA), using 300bp paired-end sequencing reads according to the manufacturer's instructions. The bioinformatics analysis was conducted using CLC Genomics software, ensuring a Phred quality score of 30. The average sequencing depth achieved was $\geq$10 000 $\times$, and the variant allele frequency (VAF) threshold was set at $\geq$ 1 %. Comparative analysis of the sequencing data, alignment optimization and variant calling were performed with software CLC Genomics Workbench version 12.

2.3.3. LAMP portable system

The new LAMP test was performed using the Doctor Vida® device with the aliquots of DNA as summarized in Fig. 3. Briefly, 5 µL of DNA sample was directly added to three reaction tubes: 1) Human EGFR gene control, 2) Specific to detect p.L858R variant and 3) Specific to detect the most frequent Exon 19 deletion, p.E746_A750del. All tubes were incubated at room temperature for 5 min and then processed using the Doctor Vida® device (STAB VIDA, Caparica, Portugal). Results were transferred and stored in real-time to an API server, through a mobile app (namely, "Doctor Vida Pocket PCR" app available for free at Google Play *Store:* https://play.google.com/store/apps/details?id=com.stabvida.dvpocket *and Apple app Store:* https://apps.apple.com/us/app/doctorvida-pocket/id1522700987*),* and automatically analyzed up to 1 h to deliver a final result that is automatically presented to the end user through the mobile app. Similar to real-time PCR's cycle threshold (Ct) value, the new LAMP test relies on a time-to-positive (Ttp) value, which is defined as the time of the assay at which the fluorescent signal exceeds the threshold set for a positive result (i.e., exceeds background level). The schematic representation of LAMP system is illustrated in Fig. 3.

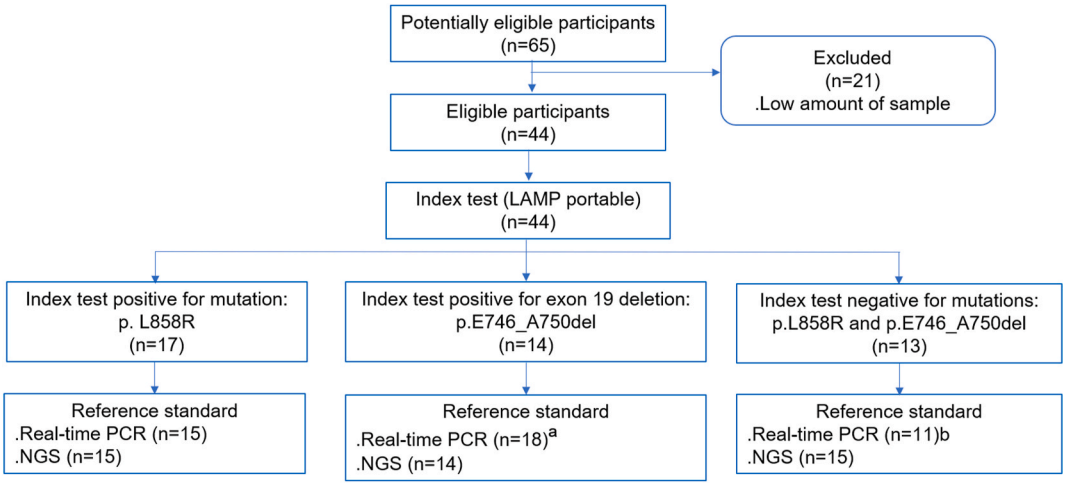

Fig. 2. Flow of participants using the LAMP (Index test) and real-time PCR and NGS (Reference standards). [a] Number of patients positive for any exon 19 deletion as real-time PCR cannot differentiate between the p.746_A750del mutation and other exon 19 deletions. [b] Number of patients negative for any exon 19 deletion and p.L858R.

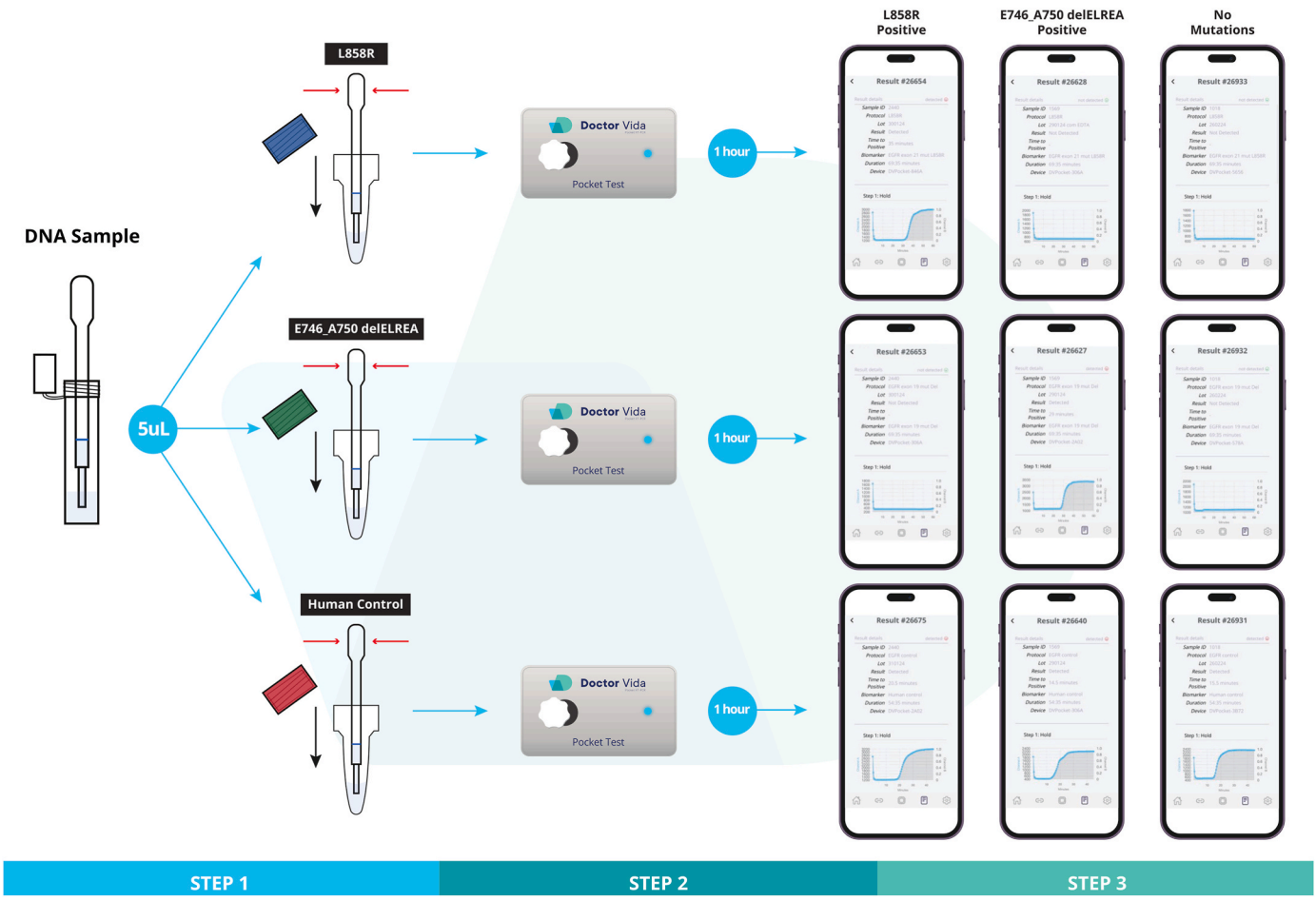

Fig. 3. Schematic representation of LAMP setup for EGFR variants testing: Step 1. Add DNA sample in each LAMP test tube to detect: p.L858R, p.E746_A750del (or E746_A750delELREA) mutations and human control. Step 2. Analysis in Doctor Vida® LAMP device. Step 3. Results obtained in Doctor Vida® mobile application for the three genotypes. Genetic mutations testing is considered valid if amplification is observed in the human control test.

2.4. Statistics

The analytical performance of the LAMP test for detecting p.L858R and p.E746_A750del variants was evaluated against reference standards, real-time PCR and NGS. All data was analyzed using the Receiver Operating Characteristic (ROC) curve analysis with a 95 % confidence interval (CI) (MedCalc Software Ltd., 2024).

2.5. Assessing the utility of LAMP portable system in lung cancer therapy

The utility of the Doctor Vida® portable LAMP testing system in clinical practice was investigated through a survey assessing its potential integration with a reference method, such as, NGS. Patients with positive results will be able to start treatment immediately, while those with negative results will undergo standard NGS (Fig. 4). The survey was distributed to medical doctors and other healthcare professionals (n = 20) involved in the treatment of lung cancer patients. These participants had the opportunity to experience the LAMP portable system demonstrations during the international research project LungCARD RISE (Grant agreement ID 734790, https://cordis.europa.eu/project/id/734790) under STAB VIDA's coordination.

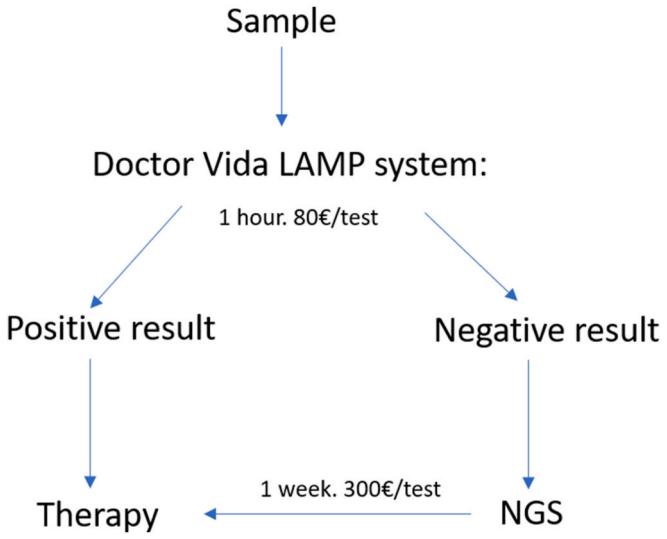

Fig. 4. Schematic representation of the proposed Doctor Vida model to use a LAMP system for EGFR mutation testing in clinical practice.

3. Results

3.1. Patients' characteristics

The analyzed patients exhibited a variety of characteristics in terms of gender, age, and cancer types. The gender distribution was 66 % female and 34 % male. Age-wise, 61 % of the patients were between 65 and 80 years old, 32 % were between 55 and 64 years old, and 7 % were between 44 and 46 years old. Regarding cancer types, 73 % of the

patients were diagnosed with adenocarcinoma.

3.2. Analytical performance of the LAMP test

The new LAMP test produced results within 1 h, demonstrating an overall accuracy of 95.45 % for detecting the p.L858R variant using both real-time PCR and NGS methods. For the p.E746_A750del variant, the test showed an overall accuracy of 100 % with NGS. Specificity with real-time PCR was 100 %; however, sensitivity could not be determined, as PCR does not identify which deletion was detected. The sensitivity, specificity, accuracy, positive predictive and negative predictive values of LAMP test for the targeted variants are summarized in Table 1.

3.3. Effect of variant allelic frequency in LAMP reaction

The samples were used directly in the LAMP reaction without any preparation to adjust the DNA concentration, nor Variant Allele Frequency (VAF). The LAMP test was conducted using 5 μL of sample, regardless of the DNA quantity, to assess the feasibility of the test without the need for DNA quantification. Table 2 presents the results of time-to-positive (Ttp) for the different variant allele frequencies. The LAMP successfully detected the somatic variants, regardless of the DNA quantity used in the reaction. The minimum quantity of variant allele detected was 0.2 ng for the p.L858R variant and 0.1 ng for the p.E746_A750del variant, respectively.

3.4. Limit of detection (LoD) for variant allele frequency (VAF)

To validate the minimum quantity of variant alleles reported in Table 2, an assay was conducted to determine the LoD for VAF. This was achieved using a dilution series of DNA from positive clinical samples within a non-mutated DNA background in triplicates. Table 3 illustrates the correlation between Ttp values and VAF at 10 %, 5 %, 1 %, and 0.5 %, using 10 ng and 20 ng of input DNA and Fig. 5 shows the lowest VAF for both variants. Using 10 ng of input DNA, a VAF of 10 % (equivalent to 2 ng) was successfully detected for the p.L858R and p.E746_A750del variants. Similarly, with 20 ng of input DNA, a VAF of 1 % (equivalent to 0.2 ng) was reliably detected for the same variants.

Table 2
Effect of quantity of variant allele, pL858R and p.E746_A750del, in the performance of LAMP system (Ttp).

Variant	Patient nº	LAMP Ttp (min)	Total DNA quantity/ reaction (ng)	Allelic variant frequency determined by NGS (%)	Quantity of variant allele/ reaction (ng)
p.L858R	17	55.0	0.4	48.6	0.2
	19	46.0	0.7	48.6	0.3
	10	29.0	0.5	77.5	0.4
	2	36.5	1.1	38.8	0.4
	7	33.5	2.2	30.8	0.7
	1	46.5	3.7	28.6	1.1
	14	32.0	5.4	39.3	2.1
	15	27.0	5.0	43.2	2.2
	9	39.5	7.5	31.8	2.4
	22	32.0	7.5	32.8	2.4
	13	32.5	8.5	40.3	3.4
	8	30.0	7.5	67.1	5.0
	35	42.5	10.6	55.8	5.9
	21	34.5	20.8	43.2	9.0
	20	35.0	71.8	26.0	18.6
p.746_A750 del	12	41.0	0.8	13.9	0.1
	30	12.5	1.0	15.4	0.2
	28	39.0	7.5	2.5	0.2
	4	14.5	2.5	8.4	0.2
	27	12.5	3.0	7.7	0.2
	31	13.5	1.2	63.9	0.7
	33	29.0	15.0	10.1	1.5
	34	15.0	9.5	21.6	2.1
	6	17.5	9.8	28.0	2.7
	16	13.5	22.2	12.8	2.8
	32	13.5	9.6	50.3	4.8
	25	20.5	7.5	76.0	5.7
	11	15.5	9.3	86.8	8.0
	24	21.5	38.4	35.9	13.8

Ttp: Time-to-Positive (min.); Quantity of mutated allele (ng) = Total DNA quantity (ng) x Allelic mutation frequency (%)/100.

4. Discussion

4.1. Analysis of discrepancies for p.L858R variant

The new LAMP test for the detection of p.L858R variant is very accurate showing, sensitivity of 100 % and specificity of 93,10 %, with two false positives. Specificity of LAMP reactions to distinguish DNA sequences with single nucleotide variants has been very challenging,

Table 1
Analytical performance of LAMP when compared with the reference standards, real-time PCR and NGS.

		Reference standard, real-time PCR						Reference standard, NGS					
		p.L858R			Exon 19 deletion[a]			p.L858R			p.E746_A750del		
		POS	NEG	TOTAL	POS	NEG	TOTAL	POS	NEG	TOTAL	POS	NEG	TOTAL
Doctor Vida LAMP	**POS**	15	2	17	14	0	14	15	2	17	14	0	14
	NEG	0	27	27	4	26	30	0	27	27	0	30	30
	TOTAL	15	29	44	18	26	44	15	29	44	14	30	44
Doctor Vida LAMP performance (CI95 %)													
Sensitivity (Coincidence rate of positive)		100.00 % (78.20 %–100.00 %)			a			100.00 % (78.20 %–100.00 %)			100.00 % (76.84 %–100.00 %)		
Specificity (Coincidence rate of negative)		93.10 % (77.23 %–99.15 %)			100.00 % (86.77 %–100.00 %)			93.10 % (77.23 %–99.15 %)			100.00 % (88.43 %–100.00 %)		
Accuracy (Total coincidence rate, PA)		95.45 % (84.53 %–99.44 %)			–			95.45 % (84.53 %–99.44 %)			100.00 % (91.96 %–100.00 %)		
Theoretical coincidence rate, Pe		0.54			–			0.54			0.57		
Kappa coefficient		0.90			–			0.90			1		
Positive Predictive Value[b]		88.24 % (66.32 %–96.62 %)			100.00 % (76.84 %–100.00 %)			88.24 % (66.32 %–96.62 %)			100.00 % (76.84 %–100.00 %)		
Negative Predictive Value[b]		100.00 % (87.23 %–100.00 %)			–			100.00 % (87.23 %–100.00 %)			100.00 % (88.43 %–100.00 %)		
Disease prevalence[b]		34.09 % (20.49 %–49.92 %)			–			34.09 % (20.49 %–49.92 %)			31.82 % (18.61 %–47.58 %)		

CI: confidence interval; NEG: negative; POS: positive.

[a] Real-time PCR method used does not specify which exon 19 deletion was detected. In contrast, the LAMP method specifically identifies the exon 19 deletion, p.E746_A750del. Consequently, it was not possible to determine the sensitivity compared to PCR.

[b] Values dependent of disease prevalence.

Table 3
Limit of Detection (LoD) for Variant Allelic Frequency (VAF) for p.L858R and p.746_A750 del.

	10 ng input DNA		20 ng input DNA	
VAF (%)	p. L858R Ttp (min)	p.E746_A750del Ttp (min)	p. L858R Ttp (min)	p.E746_A750del Ttp(min)
10	**36.5**	**13.5**		
	43	**15.5**		
	36.5	**30**		
5	**ND**	**ND**	**42.5**	**24.5**
	53	**38.5**	**35**	**17.5**
	39.5	**30.5**	**39.5**	**15**
1	**ND**	**ND**	**35.5**	**50.5**
	ND	**ND**	**47.5**	**32.5**
	ND	**ND**	**38.5**	**19**
0.5			**ND**	**30**
			ND	**ND**
			ND	**ND**

VAF: Variant Allelic Frequency; Ttp: Time-to-positive; ND: Not Detected.

especially because primers are tolerant to mismatches (Tamanaha et al., 2022). Although the new test was designed to enhance specificity by improving primers design, the occurrence of two false positives (Table 1) indicates that further investigation is needed to increase the analytical specificity, such as, the use of oligonucleotides to block the wild type amplification (Shimizu et al., 2016).

4.2. Analysis of discrepancies for exon 19 deletion

Although several exon 19 deletions have been identified in lung cancer patients (Kaneda et al., 2014; Zhao et al., 2020), the new LAMP test was designed to detect specifically the most frequent exon 19 deletion, p.E746_A750del, as shown in Figs. 1A and 6B. In the present study, it was identified four discrepant results between real-time PCR and LAMP. According to real-time PCR results, 40.9 % (n = 18/44) of the samples contained an exon 19 deletion; however, the specific deletion could not be identified as real-time PCR was unable to distinguish among exon 19 deletions. In contrast, the LAMP test specifically detected the exon 19 deletion, p.E746_A750del, in 31.8 % (n = 14/44) of the samples. The NGS analysis revealed that the four patients who were not detected by the LAMP test have an exon 19 deletion distinct from the p.E746_A750del variant (Fig. 6A), which explains why these variants were not detected by the LAMP test (Fig. 1B, . C, Fig. 6C, . D). Based on NGS results, the new LAMP portable system was able to detect the target exon 19 deletion, p.E746_A750del with 100 % specificity and sensitivity.

4.3. Analysis of limit of detection for variant allele frequency

A minimum input of 20 ng of DNA is required for the detection of a variant allele frequency of 1 % (equivalent to 0.2 ng), as demonstrated in Table 3 and Fig. 5. This result aligns with the minimum detected variant allele quantity of 0.1 and 0.2 ng observed in the analysis of clinical samples (Table 2), further supporting the assay's limit of detection.

In addition, variation in TtP values was observed among triplicates (Table 3). While LAMP can offer a general indication of template abundance through time to positivity (Ttp), it is inherently semi-quantitative rather than truly quantitative. Unlike qPCR with Taq polymerase, LAMP lacks a well-defined exponential amplification phase and exhibits notable run-to-run variability, even among nominally identical reactions. This variability can arise from factors such as pipetting inconsistencies, template quality, potential inhibitors, and subtle differences in temperature or optical calibration between instruments. Indeed, commercial LAMP platforms (e.g. those from Eiken Chemical, NEB WarmStart, Lucira, TwistDx) routinely limit claims to detection rather than precise quantification, defaulting to qPCR when rigorous measurement is required. Accordingly, any interpretation of LAMP Ttp data—especially from clinical or degraded samples—should emphasize presence/absence or broad concentration ranges, rather than absolute copy number estimation.

4.4. Utility of LAMP portable system in clinical practice

Health professionals (n = 20), comprising 65 % medical doctors and 35 % geneticists/biologists from five countries (Portugal, UK, Azerbaijan, Uzbekistan, Peru, and Serbia), unanimously agree that the proposed LAMP model is a valuable tool to be integrated into clinical practice alongside current standard routines, such as NGS (Fig. 7). Alongside feedback from health professionals, the benefits of using a portable LAMP system are clearly outlined in Table 4: a) similar analytical performance comparable to reference methods such as real-time PCR and NGS, b) results from sample to result within 1 h, c) portable and user-friendly and d) lower costs on equipment (300€) and in the test (80€).

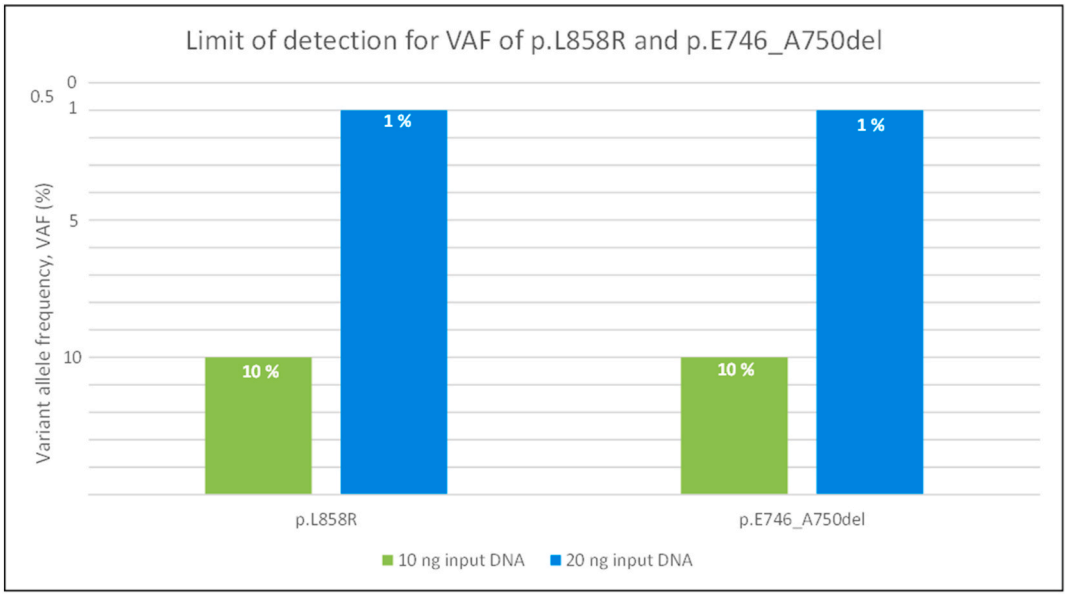

Fig. 5. Graphical representation of the minimum detectable VAF for p.L858R and p.E746_A750del variants using 10 ng and 20 ng of input DNA.

A

Patient nº	Exon 19 deletions		
	LAMP system (p.E746_A750del)	Real-time PCR (any exon 19 deletion)	NGS
3	Negative	Positive	p.L747_P753delinsS
5	Negative	Positive	p.L747_P753delinsS
26	Negative	Positive	p.E746_S752delinsV
29	Negative	Positive	p.L747_P753delinsS

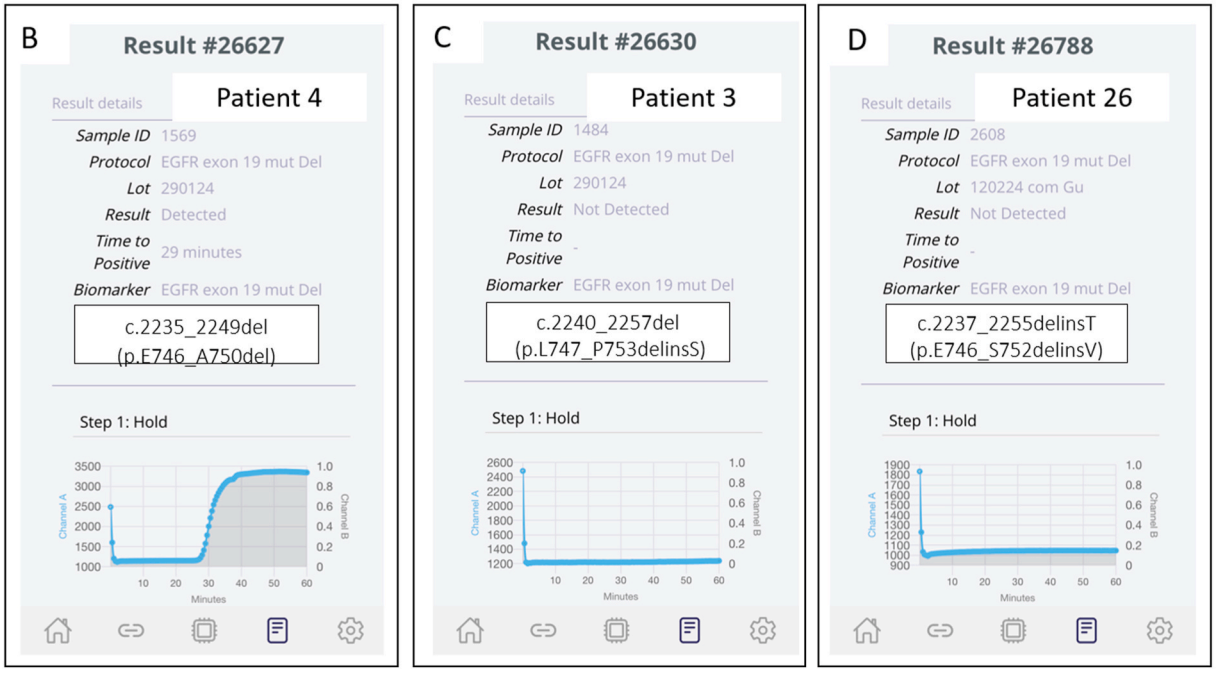

Fig. 6. (A) Patients with exon 19 deletions other than p.E746_A750del. (B), (C), (D) Examples of LAMP results for exon 19 deletion testing. (B) Patient nº 4: p. E746_A750del, (C) Patient nº 3: p.L747_P753delinsS. Similar results were observed for patients nº 5 and nº 29 (data not shown) and (D) Patient 26: p. E746_S752delinsV.

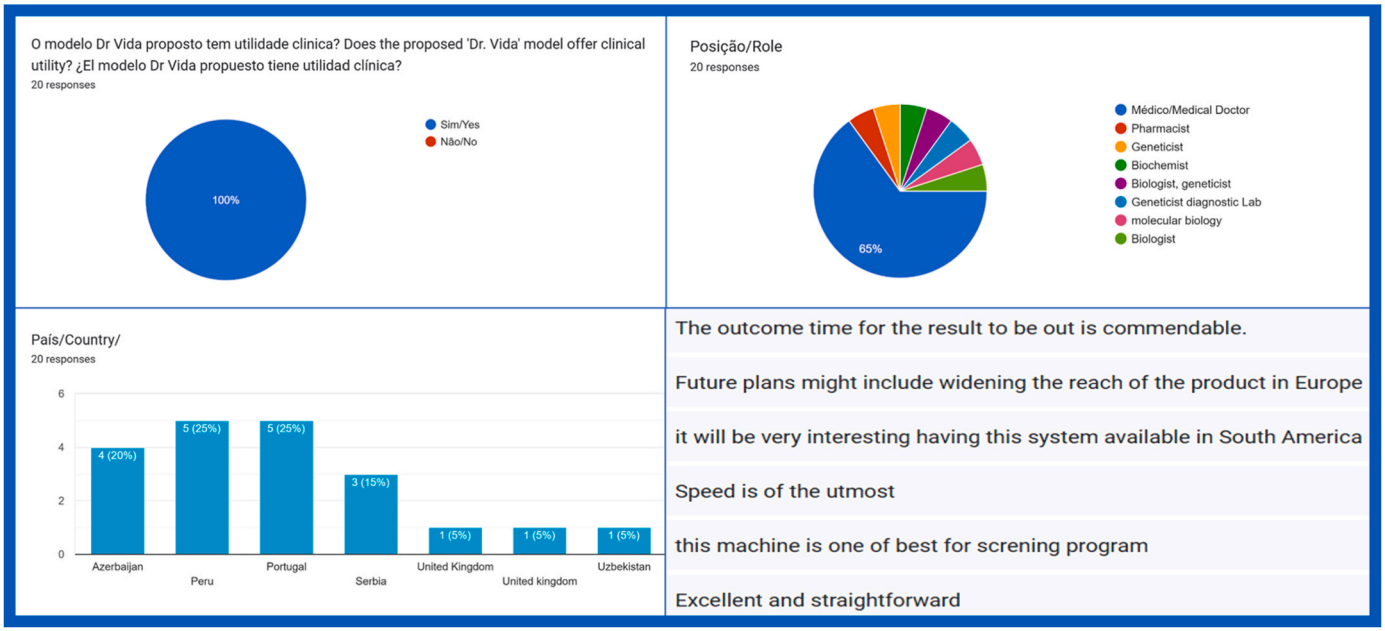

Fig. 7. Survey feedback on the utility of the Doctor Vida LAMP system in clinical practice.

Table 4
Comparison of the new LAMP portable system with reference technologies for EGFR mutation analysis.

Commercial product	Technique	Mutations in EGFR gene	Throughput	Sample to result	Process	Required resources	User experience	Test and Equipment Costs	Limit of Detection (input DNA)
cobas® EGFR Mutation Test IVD	Real time PCR	All	Multiple samples	Number of samples dependent 1–5 days	Manual	Laboratory environment	Experienced staff	Test ~ 150-200€ Equipment >20 000€	Deletion:1.4 % L858R:4 % (50 ng DNA)
theraScreen EGFR IVD	Real time PCR	All	Multiple samples	Number of samples dependent 1–5 days	Manual	Laboratory environment	Experienced staff	Test ~ 150-200€ Equipment >20 000€	5 % (requires a prior QC PCR with Ct 28–30)
Biocartis, Idylla RUO	Real time PCR	All	Single sample	1 day	Automated	Laboratory environment	User-friendly	Test ~ 150-200€ Equipment >30 000€	≥10 % neoplastic cells (5 μm FFPE tissue section)
AmoyDx® Pan Lung Cancer PCR Panel IVD	Real time PCR	All	Multiple samples	Number of samples dependent 1–5 days	Manual	Laboratory environment	Experienced staff	Test ~ 150-200€ Equipment >20 000€	1–5 % (10 ng DNA)
Illumina Pillar oncoReveal Solid Tumor Panel	Sequencing by synthesis	All	Multiple samples	Number of samples dependent 5–15 days	Manual	Laboratory environment	Experienced staff	Test ~ 500-600€ Equipment >150 000€	1 % (20–80 ng DNA)
New LAMP portable system	LAMP	p.L858R and p. E746_A750del	Single sample	~1 h	Automated	Portable. Compatible with a powerbank	User-friendly	Test ~80€ Equipment 330€	1 % (20 ng DNA)

5. Conclusions

Despite the study's limitations, including a small sample size and being conducted at a single oncology center, these findings indicate that the new LAMP portable system is a promising tool for clinical practice. To our knowledge, this is the first LAMP portable system used in an oncology center that accurately detects the most frequent variants in the EGFR gene within a cohort of NSCLC patients. Moreover, the positive feedback of healthcare professionals involved in the therapy of lung cancer patients, is very encouraging for continuing improvements in the LAMP test for detection of *EGFR* variants. Further research is ongoing in the key areas.

1. Optimization of the LAMP portable system for EGFR testing using plasma, eliminating the need for DNA purification.
2. Inclusion of additional clinical variants, such as, other exon 19 deletions and T790M variant.
3. Validation of this system across three multicenter cohorts of NSCLC patients.

In the future, integrating the Doctor Vida® LAMP system into patient care centers will enhance access to targeted therapy and expedite treatment decisions for lung cancer patients harbouring the most frequent genetic alterations variants.

CRediT authorship contribution statement

Carla Clemente: Writing – original draft, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Madalena Sequeira:** Methodology, Conceptualization. **Paulo Almeida:** Software, Data curation. **Mafalda Carvalho:** Validation. **Elnara Aliyeva:** Validation, Resources. **Sabina Mehdizadeh:** Validation, Resources. **Jamil Aliyev:** Writing – review & editing, Resources, Project administration. **Leylakhanim Melikova:** Writing – review & editing, Validation, Supervision, Resources. **Orfeu Flores:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Impact statement

This study will benefit NSCLC patients who are positive for the most frequent somatic variants in the *EGFR* gene. The results demonstrate the high analytical performance of the new LAMP portable system compared to reference methods such as NGS and real-time PCR. This manuscript advances knowledge in oncology by showcasing the feasibility of integrating the first LAMP system into the treatment decision process for NSCLC patients. By reducing time and costs, this approach accelerates access to targeted therapy for a larger number of patients.

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Conflict of interest and funding disclosure

C. Clemente, M. Sequeira, O. Flores, P. Almeida and M. Carvalho are employed by STAB VIDA Lda, the developer and manufacturer of the Doctor Vida® pocket system described in this manuscript. O. Flores is a co-inventor of a patent related to the Doctor Vida® pocket system described in this manuscript and is the founder and owner of the majority of STAB VIDA Lda.

L. Melikova, J. Aliyev, E. Aliyeva and S. Mehdizadeh have no conflicts of interest to declare.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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