DOI: 10.11134/btp.1.2026.6

UDC 577.21:575.224:57.088.1 *Original Article*

DEVELOPMENT AND VALIDATION OF A METHOD FOR DETECTING THE BRAF V600E SOMATIC MUTATION BY THE ALLELE-SPECIFIC LAMP METHOD

Khrapov E.A., Shamovskaya D.V., Smertina M.A., Obrabin I.P., Boyarskikh U.A., Filipenko M.L.*

Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
**Corresponding author: mfilipenko@gmail.com*

ABSTRACT

Detection of the somatic BRAF V600E mutation is a mandatory component of molecular stratification in melanoma, as its presence determines eligibility for BRAF and MEK inhibitor therapy [1–4]. Although qPCR- and NGS-based platforms remain the most widely used approaches for BRAF V600 mutation testing, interest in simpler, faster, and more cost-effective isothermal amplification formats persists [7, 22].

To develop and validate an allele-specific loop-mediated isothermal amplification (LAMP) assay for detection of the somatic BRAF V600E mutation in DNA extracted from tumour tissue.

An allele-specific LAMP format was employed. Analytical performance was characterised using linearised plasmid controls pV600WT and pV600E and their mixtures. Diagnostic validation was performed on 67 DNA samples extracted from FFPE melanoma blocks. The cobas 4800 BRAF V600 Mutation Test was used as the reference method.

The analytical limit of detection for mutant allele fraction was 1%, and the LoD_{59} for mutant allele copy number was 200 copies per reaction. In the clinical sample set, sensitivity of LAMP-V600E relative to AS-qPCR was 93.5% (29/31), specificity 100% (36/36), positive predictive value 100%, negative predictive value 94.7%, overall accuracy 97.0%, and Cohen's kappa coefficient 0.940. The two false-negative results were most likely attributable to FFPE-DNA degradation and the greater sensitivity of LAMP to template integrity over the comparatively longer amplification target [23, 24].

The developed allele-specific LAMP assay for BRAF V600E detection demonstrates high specificity, acceptable clinical sensitivity, and strong concordance with the reference AS-qPCR platform. The assay may be considered a rapid and technically accessible option for tissue-based BRAF testing; however, the quality and quantity of FFPE-derived DNA must be taken into account for reliable performance.

Keywords: BRAF V600E; LAMP; melanoma; FFPE; molecular diagnostics; targeted therapy.

INTRODUCTION

Melanoma is among the malignancies in which molecular stratification directly determines the choice of systemic treatment. Activating mutations in the *BRAF* gene are a defining molecular feature of cutaneous melanoma, with the p.Val600Glu (V600E) substitution being by far the most prevalent. Current clinical guidelines mandate *BRAF* V600 status assessment as a standard step in the work-up of patients with resectable and unresectable stage III–IV melanoma, since the presence of the mutation determines eligibility for targeted therapy with BRAF and MEK inhibitors [1, 2].

The clinical importance of accurate and timely *BRAF* V600E detection reflects the substantial survival benefit conferred by BRAF inhibitors and their combinations with MEK inhibitors in patients with *BRAF*-mutant melanoma. The pivotal vemurafenib trial demonstrated a statistically significant overall survival advantage over dacarbazine, and subsequent studies of the dabrafenib/trametinib and encorafenib/binimetinib combinations have established *BRAF* testing as an obligatory component of the patient pathway [3–5].

A variety of methods have been employed for BRAF V600 mutation detection in clinical specimens, including Sanger sequencing, allele-specific qPCR, next-generation sequencing (NGS), pyrosequencing, digital PCR, and immunohistochemistry. In routine practice, qPCR-based platforms and validated commercial assays have become predominant owing to their high analytical sensitivity, reproducibility, and compatibility with FFPE material. However, standard qPCR formats require thermal cycling, specialised instrumentation, and, as a rule, a relatively complex laboratory infrastructure [18–22].

Among the most promising alternative approaches are isothermal DNA amplification methods, of which loop-mediated isothermal amplification (LAMP), first described by Notomi et al. [6], is the most widely adopted. The method uses a set of four to six primers recognising multiple sites on the target, together with a strand-displacing DNA polymerase, enabling rapid DNA amplification at a constant temperature. LAMP combines high speed, technical simplicity, and versatile product detection options—including intercalating dyes and various colorimetric indicators [6–9, 11].

Despite the attractiveness of LAMP, the development of allele-specific variants for somatic point mutations remains challenging. Reliable discrimination between mutant and wild-type sequences requires careful primer design and, in many cases, the deliberate introduction of an artificial mismatch into the allele-specific primer to enhance selectivity. In recent years, several studies have demonstrated isothermal detection of *BRAF* V600E using modified LAMP, RPA–CRISPR, and RCA strategies; however, the analytical characteristics of such systems and their performance with FFPE specimens remain heterogeneous [12–16].

An additional complicating factor is the use of DNA extracted from FFPE blocks. Formalin fixation, together with the chemical and thermal effects of tissue processing, results in DNA fragmentation, chemical base modifications, and reduced amplifiability; these problems become more pro-

DOI: 10.11134/btp.1.2026.6

nounced as amplicon length increases. This is particularly relevant for LAMP, where the target region is generally longer than in qPCR [23–25].

The aim of this study was to develop and validate an allele-specific LAMP assay for detection of the somatic BRAF V600E mutation in clinical DNA samples from melanoma tissue, with evaluation of analytical performance characteristics, diagnostic efficacy, and the effect of FFPE-DNA quality on assay results.

MATERIALS AND METHODS

A DNA sample set from melanoma specimens previously collected as part of the RUSSCO Cancer Genome diagnostic project (https://www.rosoncoweb.ru/activities/cancergenome/) was used for testing. The collection comprised 36 samples negative for BRAF V600E and 31 positive samples, as determined by the reference AS-qPCR platform.

Control materials were prepared from plasmids harboring a 515-bp fragment of exon 15 of the *BRAF* gene. Two clones were generated and confirmed by Sanger sequencing: pV600WT (wild-type) and pV600E (carrying the mutation). Following linearization with the BamHI restriction endonuclease and quantification, pV600WT/pV600E mixtures were prepared at mutant allele fractions of 20, 10, 5, 2, 1, 0.5, and 0.2% at total concentrations of 10^6 and 10^5 copies/µL.

LAMP reactions (20 µL) contained 1× Gss-Sto polymerase buffer, 1.5 mM each dNTP, 0.4 µM outer primers F3/B3, 0.3 µM loop primers LB/RB, 1.6 µM inner primers FIP/BIP, template DNA, and 3 U Gss-Sto polymerase [10]. SYTO-13 intercalating dye (0.5 µM) was used for real-time fluorescence monitoring. Reactions were performed in a CFX96 Touch real-time PCR system (Bio-Rad) at 63 °C with signal acquisition every 60 seconds.

Amplification results were evaluated using the time-to-threshold (T_t) parameter. For result interpretation, the discriminatory index dT_t—defined as the difference between T_t values in the mutation-specific and WT-specific reactions—was calculated. The cobas 4800 BRAF V600 Mutation Test (Roche Diagnostics) was used as the reference method. Diagnostic performance metrics were calculated from a 2×2 contingency table against the reference AS-qPCR.

RESULTS

The design of the allele-specific LAMP system was based on a strategy in which the 3′-terminal nucleotide of the FIP-MUT primer corresponds to the mutant allele. To improve discrimination between the mutant and wild-type sequences, an artificial mismatch was introduced at the −3 position of both FIP-MUT and FIP-WT. Loop primers LB and RB were designed to accelerate amplification kinetics [8]. The complete primer sequences are listed in Table 1.

Initial validation of the two systems, LAMP-V600-WT and LAMP-V600-MUT, was performed using plasmid DNA templates (2×10^5 copies per reaction) containing either the wild-type or V600E mutant sequence. Both systems clearly discriminated WT from mutant sequence. For LAMP-V600-MUT, T_t(MUT) was 18.1 min (SD=0.97) with the mutant plasmid and 35.93 min (SD=2.18) with the wild-type plasmid; the no-template control (NTC) showed T_t values of approximately 50 min (Figure 1A). Amplification specificity was confirmed by melt curve analysis (Figure 1B).

The resulting discrimination index ($dT_t = T_t(WT) - T_t(MUT)$) was 17.83 min, which constitutes a satisfactory value. For LAMP-V600-WT, T_t(WT) was slightly higher at 20.4 min (SD=1.23), while the mutant plasmid gave T_t of 33.7 min (SD=1.91) and the NTC reached threshold at approximately 56 min. These data confirm the feasibility of V600E detection using the proposed dual-reaction system.

Despite the presence of repetitive concatenated amplicons, LAMP product melting occurs at a single temperature and generates a well-defined single peak.

In the next step, the minimum detectable mutant allele fraction was determined. Plasmid mixtures of pV600WT and pV600E at fractions of 20, 10, 5, 2, 1, 0.5, and 0.2%, as well as a pure pV600WT sample, were tested at a total concentration of 10^5 copies/µL. Each concentration level was tested in 20 replicates; positivity was defined as ≥19 reactions scoring positive for BRAF V600E. The discrimination index $dT_t = T_t(WT) - T_t(MUT)$ was calculated for each replicate pair. Given the relatively wide SD values observed during initial characterisation (approximately 2 min), the minimum acceptable discrimination index was set at 3×SD = 6 min. The minimum reliably detectable mutant allele fraction was determined to be 1%, at which T_t(MUT) = 28.2 min (SD=1.32), T_t(WT)

Table 1. Oligonucleotide primer sequences

Primer	Sequence (5'→3')
FIP-MUT	GATCCAGACAACTGTTCAAACTGAGGTGATTTTGGTCTAGCTACTGA
FIP-WT	GATCCAGACAACTGTTCAAACTGAGGTGATTTTGGTCTAGCTACTGT
BIP	TGTGGATGGTAAGAATTGAGGCTTTCTAGTAACTCAGCAGCATCTC
LB	GGACCCACTCCATCGAGAT
RB	TTCCACTGATTAAATTTTTGGC
F3	TCATGAAGACCTCACAGTAAAA
B3	TACTATAGTTGAGACCTTCAATGAC

Note. FIP-MUT and FIP-WT are allele-specific inner primers (F2+F1c); their 3′-terminal nucleotide of the F2 portion corresponds to the mutant (T→A) or wild-type BRAF allele, respectively. To enhance allelic discrimination, an artificial mismatch was introduced at position −3 relative to the 3′-end of both allele-specific primers. All remaining primers (BIP, LB, RB, F3, B3) are shared between both reaction systems.

DOI: 10.11134/btp.1.2026.6

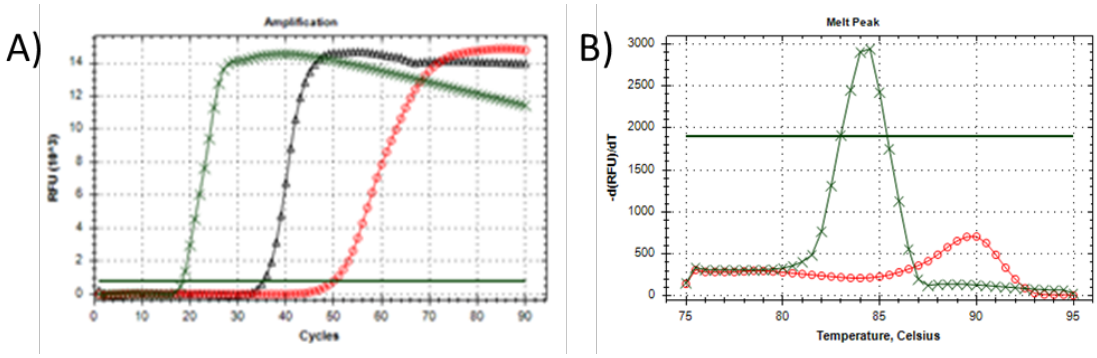

Figure 1. A) Real-time LAMP amplification curves: mutant DNA is shown by green crosses, wild-type DNA by black triangles, and the no-template control (NTC) by red diamonds. B) Melt curves of LAMP products: mutant DNA is shown by green crosses, the NTC by red circles.

= 36.1 min (SD=2.25), and mean T_t(WT) − T_t(MUT) = 7.8 min. The corresponding LAMP-V600-WT system gave T_t = 21.3 min (SD=1.9), yielding dT_t = 5.2 min. On this basis, a sample was classified as positive for V600E if the dT_t value calculated from the paired LAMP-V600-MUT and LAMP-V600-WT reactions was less than 5 min.

The LoD_{59} for mutant allele copy number was determined by serial dilution of a 1% mutant plasmid standard at 50, 100, 200, 500, and 1000 copies of mutant allele per reaction, with 20 technical replicates per concentration level. The LoD_{59} was 200 copies of mutant allele per reaction, which at 1% VAF corresponds to 20,000 human genome equivalents per reaction (approximately 66 ng). Accordingly, a minimum input of approximately 132 ng of tumour DNA is required to reliably perform the assay.

A critical operational parameter for diagnostic LAMP is reaction duration, as the amplification time is strictly limited by the risk of non-specific amplification in no-template controls (NTC). Prolonged incubation increases the probability of false-positive results and consequent misinterpretation. Since no increase in NTC fluorescence was observed within the first 50 min, and the maximum T_t for positive samples at the LoD_{59} level was 30 min, the diagnostic LAMP run duration was set at 45 min.

Diagnostic validation was performed on 67 DNA samples from FFPE melanoma blocks, using the cobas 4800 BRAF V600 Mutation Test (AS-qPCR) as the reference standard. The results are summarized in Table 2.

Table 2. Results of BRAF V600E mutation testing in 67 melanoma FFPE DNA samples

	AS-qPCR (+)	AS-qPCR (−)	Total
LAMP-V600E (+)	29	0	29
LAMP-V600E (−)	2	36	38
Total	31	36	67

The clinical sensitivity of LAMP-V600E was 93.5% and specificity was 100% relative to AS-qPCR (Table 3). Cohen's kappa coefficient of 0.940 indicates near-perfect agreement between the two methods. Positive and negative predictive values were 100% and 94.7%, respectively.

DISCUSSION

This study presents the development and validation of an allele-specific LAMP assay for detection of the somatic *BRAF* V600E mutation. A key feature of the proposed approach is the use of two parallel reactions—targeting the mutant and wild-type sequences, respectively—with result interpretation based on the difference in time-to-threshold values (dT_t). This dual-reaction scheme is well suited to allele-specific LAMP, as it accounts not only for the presence of a signal but also for the kinetic difference between specific early amplification and late non-specific amplification, and reflects the total amount of human DNA input. Data obtained with plasmid models demonstrated robust discrimination between mutant and wild-type sequences, and the introduction of an artificial mismatch into the FIP primers appears to have contributed substantially to improved selectivity [6–9, 11].

From an analytical standpoint, the assay achieved a limit of detection of 1% mutant allele fraction and an LoD_{95} of 200 mutant allele copies per reaction. For tissue-based tumour analysis this represents an acceptable level of sensitivity, particularly in settings with limited instrumental infrastructure, although further development would be needed to translate the assay into a qualitative instrument-free format. At the same time, these figures indicate that the assay is not designed for ultra-sensitive low-allele-fraction applications—such as plas-

Table 3. Diagnostic performance of the LAMP-V600E assay relative to AS-qPCR

Parameter	LAMP-V600E
Sensitivity	93.5% (29/31; 95% CI 78.6–99.2%)
Specificity	100% (36/36; 95% CI 90.3–100%)
Positive predictive value (PPV)	100% (29/29; 95% CI 88.3–100%)
Negative predictive value (NPV)	94.7% (36/38; 95% CI 82.7–98.5%)
Overall accuracy	97.0% (65/67; 95% CI 89.8–99.2%)
Cohen's kappa coefficient (κ)	0.940 (95% CI 0.86–1.00)

DOI: 10.11134/btp.1.2026.6

ma-based liquid biopsy—where digital PCR and certain specialised qPCR formats achieve substantially lower LoD values for mutant allele fraction [22, 26].

In the clinical series of 67 FFPE melanoma specimens, LAMP-V600E demonstrated 93.5% sensitivity and 100% specificity against the validated cobas 4800 AS-qPCR reference. The κ value of 0.940 indicates near-perfect inter-method agreement. Particularly noteworthy is the complete absence of false-positive results in the evaluated series, given that analytical specificity is of critical importance for assays that support decisions on BRAF/MEK inhibitor therapy [1–3].

The most plausible explanation for the two false-negative results is the quality of the source FFPE-DNA. This is consistent with both the characteristics of the two discordant samples and published evidence demonstrating that FFPE-DNA is subject to pronounced fragmentation, chemical base modifications, and reduced amplifiability, with these effects becoming more severe as amplicon length increases. In FFPE specimens, the average length of amplifiable fragments is often below 300 bp, and longer amplicons lose reproducibility far more rapidly than the short qPCR targets [23–25]. LAMP is therefore inherently more susceptible to template degradation than short-amplicon AS-qPCR.

A comparison with other published isothermal approaches to *BRAF* V600E detection places the performance of the present assay in an intermediate but clinically meaningful position. Papadakis et al. developed a portable real-time colorimetric qcLAMP platform and demonstrated, using purified tissue DNA, the ability to detect BRAF V600E at 0.01% allele fraction in 100 ng of total DNA. In a blinded evaluation of 12 clinical samples, complete concordance with Sanger sequencing and ddPCR was achieved, with four out of five positive samples detected in under 30 min. However, when applied directly to FFPE sections, reproducibility dropped markedly: a positive result was obtained in only 2 of 8 replicates for DNA with 1% mutant allele, whereas 10–50% allele fractions were detected considerably more reliably [13]. While this study demonstrates extremely high analytical sensitivity on purified DNA, it also confirms that real-world FFPE material remains a critical limitation.

Varona et al. described a molecular-beacon LAMP assay for the detection of *BRAF* V600E in circulating tumour DNA (ctDNA) from plasma. According to the published data and the characteristics cited by the authors, the assay was primarily designed for compatibility with liquid biopsy material and qualitative endpoint-based readout rather than for achieving record-low mutant allele fractions. The study emphasised ctDNA isolation and detection from plasma and the applicability of the approach to allelic discrimination [12, 14]. Compared with this work, the present assay offers a more direct adaptation to tissue-derived FFPE-DNA and formally verified clinical performance metrics for melanoma, but has not yet been adapted for plasma-based applications.

A further example of isothermal *BRAF* V600E detection is provided by Etemadzadeh et al., who employed an RPA/CRISPR-Cas12a combination. This format claimed a limit of detection of 2% and a total assay time of approximately 75 min. Compared with this approach, the LAMP system reported here offers comparable selectivity, slightly better sensitivity for the mutant allele fraction (1% versus 2%), and a shorter practical assay time, without requiring CRISPR reagents or an additional post-amplification signal cascade [15].

Dekaliuk et al. described a distinct isothermal strategy combining rolling circle amplification (RCA) with FRET-based hybridisation detection. This approach provided an analytical range of 75 fM to 4.5 pM (approximately 4.5×10^5 to 2.7×10^7 copies) and was primarily oriented towards highly specific analytical discrimination of the V600E variant. Compared with this system, the present assay is instrumentally less complex and more amenable to integration into routine molecular diagnostics, although RCA-FRET remains an interesting example of a high-specificity isothermal platform for point mutations [16].

Most recently, Moráňová et al. (2025) proposed an electrochemical readout LAMP format for BRAF V600E targeting liquid biopsy applications. While this approach highlights high selectivity and promising potential for rapid mutant DNA analysis, it requires specialised electrochemical detection equipment and, at this stage, appears less amenable to implementation in a standard oncology laboratory than fluorescence-based real-time LAMP performed on a conventional thermocycler [17].

Taken together, compared with published isothermal approaches, the present system does not claim a record analytical sensitivity, but offers an important practical advantage: it employs classical real-time monitoring on a widely available instrument, requires no complex probe constructs or specialised devices, and delivers high diagnostic specificity as demonstrated specifically on FFPE melanoma DNA. In practical terms, this makes the assay attractive for laboratories requiring a rapid and cost-effective tissue-based BRAF V600E test, rather than a universal ultra-sensitive platform for all sample types.

Strengths of the study include the rational design of allele-specific primers, the use of plasmid reference standards for analytical sensitivity characterisation, the availability of a clinical validation cohort, and comparison against a well-characterised IVD reference. Limitations include the relatively small clinical sample size, the exclusive focus on the BRAF V600E variant without coverage of V600K or V600R, and the absence of orthogonal verification of discordant samples by ddPCR or NGS. Future work should investigate inter-run reproducibility, assay robustness across the spectrum of FFPE-DNA quality variation, and the feasibility of reducing the length of the amplification target.

CONCLUSIONS

The developed LAMP assay demonstrates high specificity and acceptable sensitivity for routine BRAF V600E diagnostics. The analytical LoD_{95} is 200 copies of mutant allele per reaction (1% VAF); the assay requires ≥132 ng of tumour DNA input, is completed in under 45 minutes, and can be implemented in resource-limited laboratories provided that input DNA quality is controlled.

All discordant results were associated with pronounced template DNA degradation, attributable to the longer LAMP amplification target relative to the short amplicons used in qPCR.

DOI: 10.11134/btp.1.2026.6

FUNDING

This work was supported by the Russian state-funded project for ICBFM SB RAS (grant number 125012300674-9).

AUTHOR CONTRIBUTION

E.A. Khrapov: LAMP optimisation and clinical sample validation. D.V. Shamovskaya: LAMP clinical sample validation. M.A. Smertina: control experiments. I.P. Oscorbin: LAMP experimental design. U.A. Boyarskikh: LAMP primer design. M.L. Filipenko: study concept, manuscript writing and editing.

REFERENCES

1. Amaral T., Ottaviano M., Arance A., et al. Cutaneous melanoma: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up // Annals of Oncology. – 2025 Jan; – Vol. 36. – No. 1. – P.10–30 // doi: 10.1016/j.annonc.2024.11.006.

2. Swetter S.M., Johnson D.B., Albertini M.R., et al. NCCN Guidelines Insights: Melanoma: Cutaneous, Version 2.2024 // Journal of the National Comprehensive Cancer Network. – 2024 May; – Vol. 22. – No. 5. – P.290–298 // doi: 10.6004/jnccn.2024.0036.

3. Chapman P.B., Hauschild A., Robert C., et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation // New England Journal of Medicine. – 2011 Jun; – Vol. 364. – No. 26. – P.2507–2516 // doi: 10.1056/NEJMoa1103782.

4. Robert C., Grob J.J., Stroyakovskiy D., et al. Five-year outcomes with dabrafenib plus trametinib in metastatic melanoma // New England Journal of Medicine. – 2019 Aug; – Vol. 381. – No. 7. – P.626–636 // doi: 10.1056/NEJMoa1904059.

5. Dummer R., Ascierto P.A., Gogas H.J., et al. Encorafenib plus binimetinib versus vemurafenib or encorafenib in patients with BRAF-mutant melanoma: a multicentre, open-label, randomised phase 3 study // Lancet Oncology. – 2018 May; – Vol. 19. – No. 5. – P.603–615 // doi: 10.1016/S1470-2045(18)30142-6.

6. Notomi T., Okayama H., Masubuchi H., et al. Loop-mediated isothermal amplification of DNA // Nucleic Acids Research. – 2000 Jun; – Vol. 28. – No. 12. – P.e63 // doi: 10.1093/nar/28.12.e63.

7. Notomi T., Mori Y., Tomita N., Kanda H. Loop-mediated isothermal amplification (LAMP): principle, features, and future prospects // Journal of Microbiology. – 2015 Jan; – Vol. 53. – No. 1. – P.1–5 // doi: 10.1007/s12275-015-4656-9.

8. Nagamine K., Hase T., Notomi T. Accelerated reaction by loop-mediated isothermal amplification using loop primers // Molecular and Cellular Probes. – 2002 Jun; – Vol. 16. – No. 3. – P.223–229 // doi: 10.1006/mcpr.2002.0415.

9. Tomita N., Mori Y., Kanda H., Notomi T. Loop-mediated isothermal amplification (LAMP) of gene sequences and simple visual detection of products // Nature Protocols. – 2008 May; – Vol. 3. – No. 5. – P.877–882 // doi: 10.1038/nprot.2008.57.

10. Oscorbin I.P., Belousova E.A., Boyarskikh U.A., Zakabunin A.I., Khrapov E.A., Filipenko M.L. Derivatives of Bst-like Gss-polymerase with improved processivity and inhibitor tolerance // Nucleic Acids Research. – 2017 Sep; – Vol. 45. – No. 16. – P.9595–9610 // doi: 10.1093/nar/gkx645.

11. Shirshikov F.V., Bespyatykh J.A. Loop-mediated isothermal amplification: from theory to practice // Russian Journal of Bioorganic Chemistry. – 2022 Nov; – Vol. 48. – No. 6. – P.1159–1174 // doi: 10.1134/S106816202206022X.

12. Varona M., Anderson J.L. Advances in mutation detection using loop-mediated isothermal amplification // ACS Omega. – 2021 Feb; – Vol. 6. – No. 5. – P.3463–3469 // doi: 10.1021/acsomega.0c06093.

13. Papadakis G., Pantazis A.K., Fikas N., et al. Portable real-time colorimetric LAMP-device for rapid quantitative detection of nucleic acids in crude samples // Scientific Reports. – 2022 Mar; – Vol. 12. – No. 1. – P.3775 // doi: 10.1038/s41598-022-06632-7.

14. Varona M., Eitzmann D.R., Pagariya D., Anand R.K., Anderson J.L. Solid-phase microextraction enables isolation of BRAF V600E circulating tumour DNA from human plasma for detection with a molecular beacon loop-mediated isothermal amplification assay // Analytical Chemistry. – 2020 Feb; – Vol. 92. – No. 4. – P.3346–3353 // doi: 10.1021/acs.analchem.9b05323.

15. Etemadzadeh A., Salehipour P., Movahedi Motlagh F., et al. An optimized CRISPR/Cas12a assay to facilitate the BRAF V600E mutation detection // Journal of Clinical Laboratory Analysis. – 2024 Nov; – Vol. 38. – No. 21. – P.e25101 // doi: 10.1002/jcla.25101.

16. Dekaliuk M., Qiu X., Troalen F., Busson P., Hildebrandt N. Discrimination of the V600E mutation in BRAF by rolling circle amplification and Förster resonance energy transfer // ACS Sensors. – 2019 Oct; – Vol. 4. – No. 10. – P.2786–2793 // doi: 10.1021/acssensors.9b01420.

17. Moráňová L., Strmisková J., Ondrašková K., et al. Electrochemical detection of BRAF V600E mutation in a liquid biopsy format: LAMPing the way to an improved diagnostics and treatment // Advanced Materials Technologies. – 2025 Jul; – Vol. 10. – No. 7. – P.2401404 // doi: 10.1002/admt.202401404.

18. Lopez-Rios F., Angulo B., Gomez B., et al. Comparison of testing methods for the detection of BRAF V600E mutations in malignant melanoma: pre-approval validation study of the companion diagnostic test for vemurafenib // PLOS ONE. – 2013 Jan; – Vol. 8. – No. 1. – P.e53733 // doi: 10.1371/journal.pone.0053733.

19. Mourah S., Denis M.G., Narducci F.E., et al. Detection of BRAF V600 mutations in melanoma: evaluation of concordance between the cobas 4800 BRAF V600 Mutation Test and the methods used in French National Cancer Institute (INCa) platforms in a real-life setting // PLOS ONE. – 2015 Mar; – Vol. 10. – No. 3. – P.e0120232 // doi: 10.1371/journal.pone.0120232.

20. Anderson S., Bloom K.J., Vallera D.U., et al. Multisite analytic performance studies of a real-time polymerase chain reaction assay for the detection of BRAF V600E mutations in formalin-fixed, paraffin-embedded tissue specimens of malignant melanoma // Archives of Pathology & Labora-

DOI: 10.11134/btp.1.2026.6

tory Medicine. – 2012 Nov; – Vol. 136. – No. 11. – P.1385–1391 // doi: 10.5858/arpa.2011-0505-OA.

21. Halait H., Demartin K., Shah S., et al. Analytical performance of a real-time PCR-based assay for V600 mutations in the BRAF gene used as the companion diagnostic test for the novel BRAF inhibitor vemurafenib in metastatic melanoma // Diagnostic Molecular Pathology. – 2012 Mar; – Vol. 21. – No. 1. – P.1–8 // doi: 10.1097/PDM.0b013e31823b216f.

22. Vanni I., Tanda E.T., Spagnolo F., et al. The current state of molecular testing in the BRAF-mutated melanoma landscape // Frontiers in Molecular Biosciences. – 2020 Nov; – Vol. 7. – P.113 // doi: 10.3389/fmolb.2020.00113.

23. Dietrich D., Uhl B., Sailer V., et al. Improved PCR performance using template DNA from formalin-fixed and paraffin-embedded tissues by overcoming PCR inhibition // PLOS ONE. - 2013 Oct; - Vol. 8. - No. 10. - P.e77771 // doi: 10.1371/journal.pone.0077771.

24. Kong H., Zhu M., Cui F., Wang S., Gao X., Lu S., et al. Quantitative assessment of short amplicons in FFPE-derived long-chain RNA // Scientific Reports. - 2014 Sep; - Vol. 4. - P.7246 // doi: 10.1038/srep07246.

25. Chat-Uthai N., Vejvisithsakul P., Udommethaporn S., et al. Development of ultra-short PCR assay to reveal BRAF V600 mutation status in Thai colorectal cancer tissues // PLOS ONE. - 2018 Jun; - Vol. 13. - No. 6. - P.e0198795 // doi: 10.1371/journal.pone.0198795.

26. Pinzani P., Salvianti F., Cascella R., et al. Allele-specific TaqMan-based real-time PCR assay to quantify circulating BRAFV600E mutated DNA in plasma of melanoma patients // Clinica Chimica Acta. - 2010 Nov; - Vol. 411. - No. 17-18. - P.1319–1324 // doi: 10.1016/j.cca.2010.05.024.