Diagonal Bio - Reinventing LAMP

Highlights

- Diagonal Bio's product pipeline contains of LAMPlify® and Panviral® platforms for pathogen detection in an 8-parallel test format.
- The core patented technology taps into the ultra-fast speed and convenience of the Loop-mediated Isothermal Amplification (LAMP), and the specificity and robustness of Electrochemical detection.
- As a part of Diagnostic Performance Evaluation of the system, over 500 tests have been assessed with 7 different genetic markers, from 6 different pathogens.
- The technology shows impressive overall Accuracy of 99.6%, Sensitivity of 100%, and Specificity of 99.5%.

Abstract

In an era where the pace of diagnostics is paramount, Diagonal Bio's innovative technology redefines the expectations from Nucleic Acid Amplification Tests (NAATs). LAMPlify®, the company's flagship offering, capitalizes on the intrinsic speed and convenience of the Loop-mediated Isothermal Amplification (LAMP) methodology, but transcends its traditional limitations. By harnessing the specificity and robustness of electrochemical detection, LAMPlify® eliminates the need for purifying DNA or RNA from biological samples, achieving unparalleled performance without the wait. With accuracy in line with the gold standard PCR, and an assay time of approximately 15 minutes, this breakthrough paves the way for rapid, point of care diagnostics, positioning Diagonal Bio at the forefront of the medical technology industry.

DIAGONAL BIO

Introduction to NAAT

The landscape of molecular diagnostics has been predominantly shaped by the advent and implementation of NAATs. These have formed the backbone of pathogen detection, genetic sequencing, and several applications critical to advancing medical science and public health. While traditional Polymerase Chain Reaction (PCR) methods have set the standard for accuracy and specificity, they come with a significant trade-off in terms of speed and operational agility, often requiring lengthy turn-around times that prevent rapid decision-making.

Rapid NAATs, like LAMP, have been developed as faster alternatives to PCR but have historically been viewed as not competitive enough. The necessity for extraction and purification of DNA or RNA from complex samples has been a persistent bottleneck for all NAATs. While the highly robust amplification reaction behind LAMP overcomes this need, the traditional means of visualizing the results from the LAMP reaction rely on optical detection including fluorescence and colour-based detection. These optical detection methods are impeded by the background impurities, compromising the test's accuracy and efficiency.

Two decades after the introduction of LAMP, Diagonal Bio enhanced the LAMP technology with a novel electrochemical approach through its LAMPlify® and Panviral® systems – for applications within non-regulated and human diagnostics market segments, respectively. The core technology taps into the inherent speed, agility, and robustness of LAMP, while leveraging the high accuracy of electrochemical detection and bypassing the costly or complex requirements of optical detection. By probing the molecular electronic states in the reaction, Diagonal Bio's diagnostic products allow for detection of DNA or RNA directly from complex matrices, providing a clear, accurate reading free from the interference of sample impurities. LAMPlify® marks the dawn of a new era in NAATs — where speed, accuracy, and operational efficiency converge to elevate diagnostic capabilities to unprecedented levels.

Birth of an Ultra-rapid, Real Time, Semi-Quantitative NAAT

During technology development and optimisation, the early experimental setups (Fig 1A.) comprised of a repurposed thermal cycler that maintained constant temperature of the incubated sample vials. For electrochemical analysis, proprietary thin-film electrodes inserted in the sample vials were connected to a standard multi-potentiostat (an electrochemical workstation) controlled by a computer. These experiments were performed with Actin DNA as the model gene of interest.

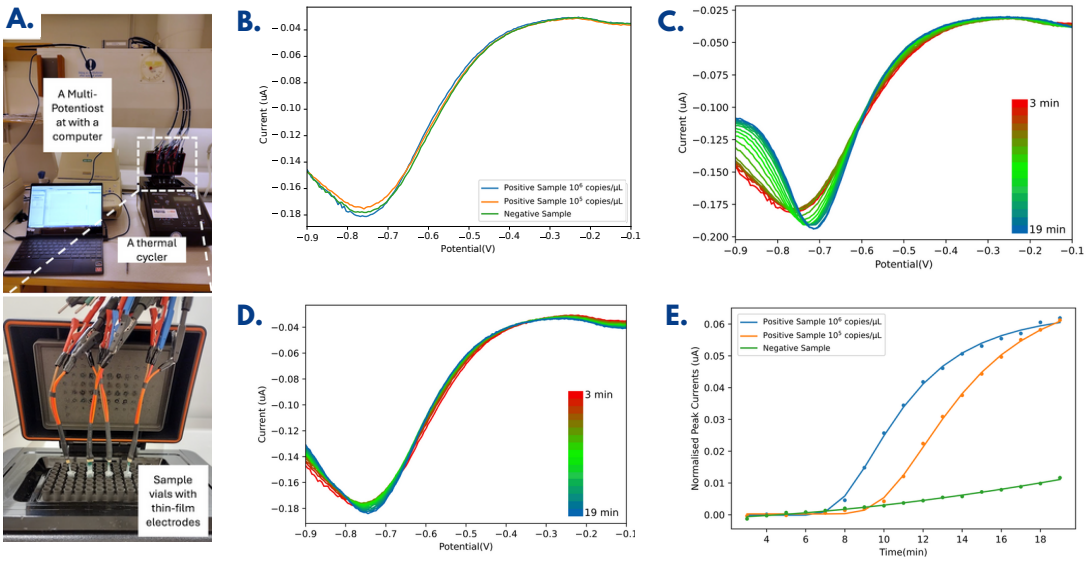

Fig 1. Diagonal Bio's technology for ultra-fast NAAT. A) Early experimental setups. Voltammetry plots for positive and negative samples with Actin DNA as the model gene of interest, showing current response at B) the beginning of the amplification reaction, and the time evolution of the voltammetry plots in C) presence and D) absence of gene of interest.

In addition to the i.) sample or gene of interest, ii.) necessary enzymes and iii.) raw material for the amplification reaction, the reaction mix in the sample vials also contains iv.) gene-specific probes called primers, and v.) a pH indicator. As shown in Fig 1B., using the electrochemical technique of voltammetry, electrical potential was applied to probe the molecular electronic states of the pH indicator in the reaction mix. As DNA amplification proceeds, protons are released as a by-product of the reaction, which causes the pH of the solution to drop and alters the molecular electronic states of the pH indicator, thereby altering the electrochemical readings. The result is a real-time, precise measurement of the amplification process, observed as a shifting valley in the voltammetric plot in Fig 1C. Conversely a negative sample, in which no gene of interest is present, displays no major position or shape changes, as shown in Fig 1D.

This approach negated the need for sample purity, as the electrochemical signals could cut through the noise, identifying the progress of DNA amplification without interference from other substances. By focusing on the position of the valley in the voltammetry plots, positive and negative samples could be easily distinguished, as evident in Fig 1E. Further, the speed of the pH change correlated with the quantity of the target gene present. This not only confirmed the presence of the target DNA but also provided an estimation of its initial amount, crucial for semi-quantitative diagnostics.

LAMPlify® and Panviral® – The User-Centric Product Design

With focus on functionality, user-centric interface, and user-safety, LAMPlify® and Panviral® were designed as a compact benchtop yet portable instrument, with an intuitive touchscreen interface that guides users through each step, making sophisticated diagnostics accessible to all. Internally, a combination of microcontrollers, potentiostats and a heat source maintains optimal temperature for the reaction while monitoring its progress. The instrument's portable nature ensures adaptability to various environments, from high-tech labs to field applications – thus facilitating applications of Diagonal Bio's innovation in both non-regulated and human diagnostics market segments.

At the heart of the system lies the cartridge, a sleek component housing eight individual vials paired with precise thin-film electrodes. This multi-channel approach means that up to eight tests can be run simultaneously, offering efficiency and adaptability to varied requirements outside central diagnostic laboratories.

Fig 2. Product design for LAMPlify® and Panviral® product lines, along with the cartridges.

Performance with Biological Samples

Diagonal Bio's LAMPlify® system has been rigorously tested with biological samples, demonstrating its robustness and reliability. One of the most compelling validations has been performed with experiments involving viruses including the SARS-CoV-2 virus.

For safety reasons, inactivated virus samples were used. The samples were used directly as the LAMPlify® and Panviral® systems do not need RNA (or DNA) purification. The data visualization was enabled by our proprietary algorithm that translated the electrochemical signals into easily interpretable results. The graphical plots provided by the LAMPlify® system allowed for immediate real-time understanding of each sample's status.

As shown in Fig 3, negative samples displayed no amplification signal, while the positive samples presented a distinct amplification pattern. Further, samples with higher virus concentrations reached detection thresholds more quickly than those with lower concentrations. This rapid detection, typically in approximately a 15 minute window, underscores the system's potential in providing timely critical data in a variety of applications.

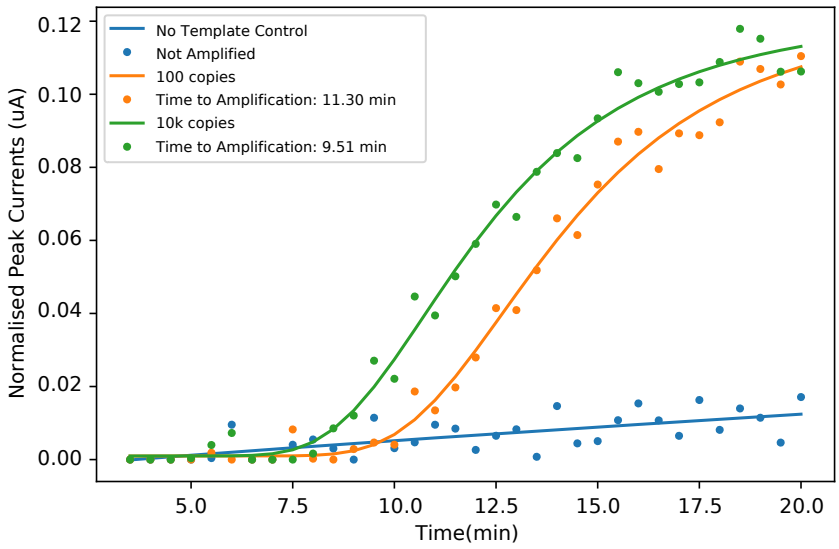

Fig 3. Performance of LAMPlify with inactivated SARS-CoV-2 virus, without the need of any RNA purification.

Performance with Biological Samples

The developed systems were evaluated for diagnostic performance with 7 different genetic markers belonging to various pathogens, including SARS-CoV2, IAV (Influenza A virus), IBV (Influenza B virus), H3N8 (Influenza A virus subtype H3N8), EHV1 (Equid alphaherpesvirus 1), and EHV2 (Equid alphaherpesvirus 4) viruses using eleven different instruments. Table 1 shows cumulative performance analysis of Diagonal Bio's diagnostic systems. For this purpose, testing was performed under controlled laboratory conditions with synthetic or inactivated target samples between October 2023 until February 2024, and a total of 511 samples were analysed, and a result was considered positive if it displayed amplification within 18 minutes. The performance evaluation demonstrated an overall accuracy of 99.6 %. Further, the tests were 100 % sensitive and 99.5 % specific. The average time of detection for positive samples containing 10,000 copies/µL of target was 11 min, while the limit of detection was even better.

		Detection with LAMPlify®		
		Positive Result	Negative Result	
Actual	Positive Samples	142	0	100% Sensitivity
	Negative Samples	2	367	99.5% Specificity
		98.6% Precision	100% Negative Predictive Value	99.6% Accuracy

Table 1. Cumulative performance evaluation of 11 LAMPlify® devices across assays for 7 different genetic markers.

These analytical specifications are in line with previously reported results from Diagonal Bio's 2022 collaborative study with Prof. Patrik Medstrand on 234 patient samples from the Skåne University Hospital biobank, for detection of for SARS-CoV-2, IAV, IBV, and RS virus, that demonstrated an accuracy of 98.7 %, specificity of 99.2 %, and sensitivity of 98.3 % when compared to PCR. This does not just demonstrate an outstanding diagnostic performance of the system, but also positions the LAMPlify® and Panviral® systems as highly competitive to the state-of-the-art PCR products.

Conclusion

Diagonal Bio's LAMPlify® and Panviral® systems represent a quantum leap in diagnostic technology. Tapping into LAMP and electrochemical detection, their ability to deliver ultra-fast results without the prerequisite of DNA or RNA purification sets a new industry standard. The system's accuracy, sensitivity and specificity are not compromised by speed, ensuring that each analysis, each decision based on its results, is as reliable as it is swift.

As Diagonal Bio is on its way to commercialise LAMPlify®, the focus is not just to broadly launch this ground-breaking innovation, but also to support market penetration with a suite of primers for various diagnostic segments. The impact is already tangible as the current users have begun to experience the benefits of rapid, onsite testing. Diagonal Bio stands at the threshold of a new era in diagnostics, ready to empower health professionals and researchers with a tool that redefines possibilities for ultra-fast detection of pathogens.