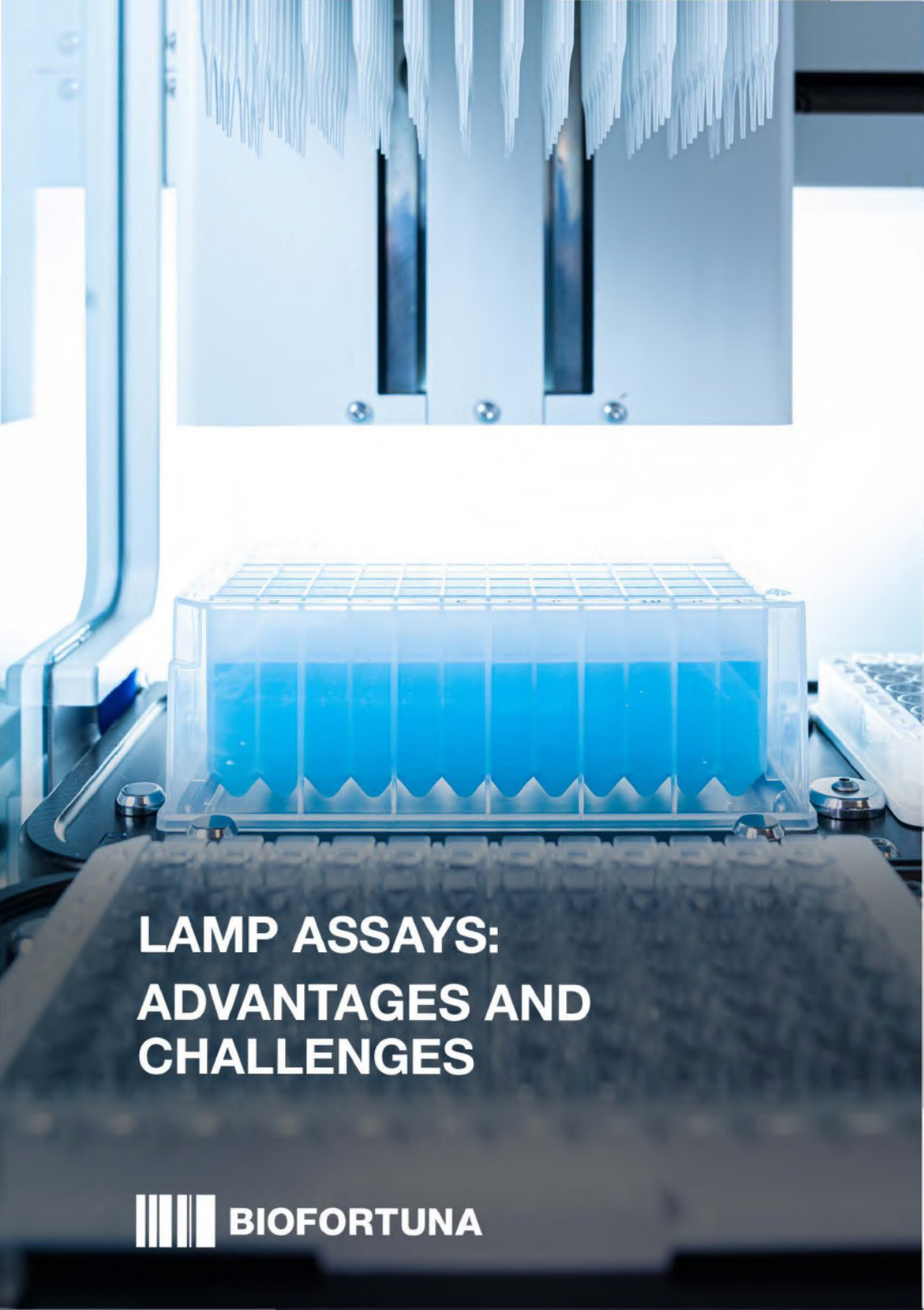


A close-up photograph of a laboratory instrument, likely a liquid handler or plate reader, processing a multi-well plate. The plate is filled with a vibrant blue liquid. Above the plate, a series of vertical tubes or nozzles are visible, suggesting a dispensing or aspiration process. The background is a clean, light blue, emphasizing the scientific and technological nature of the scene.

LAMP ASSAYS: ADVANTAGES AND CHALLENGES



BIOFORTUNA

THE EVOLUTION OF NUCLEIC ACID AMPLIFICATION TECHNIQUES

Since its development in the late 1980's, PCR has been considered the gold standard for DNA amplification for molecular analysis. However, the requirement for dedicated and costly instrumentation to provide appropriate temperature cycling control has limited the use of PCR mainly to laboratory settings.

This bottleneck has led to the development of several non-PCR based techniques that operate at a constant temperature and have simple workflow protocols that can be adopted for Point of Care use, moving molecular diagnostics away from centralised laboratories.

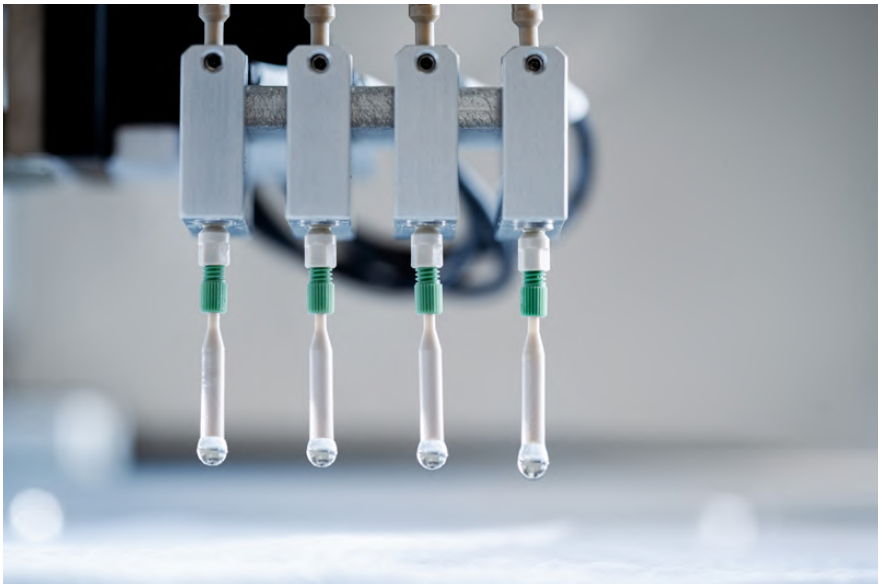
These isothermal methods include strand displacement amplification (SDA), rolling circle amplification (RCA), nucleic acid sequence-based amplification (NASBA), helicase-dependent amplification (HDA), transcription-mediated amplification (TMA), recombinase polymerase amplification (RPA) and several others in addition to Loop-Mediated Isothermal Amplification (LAMP).

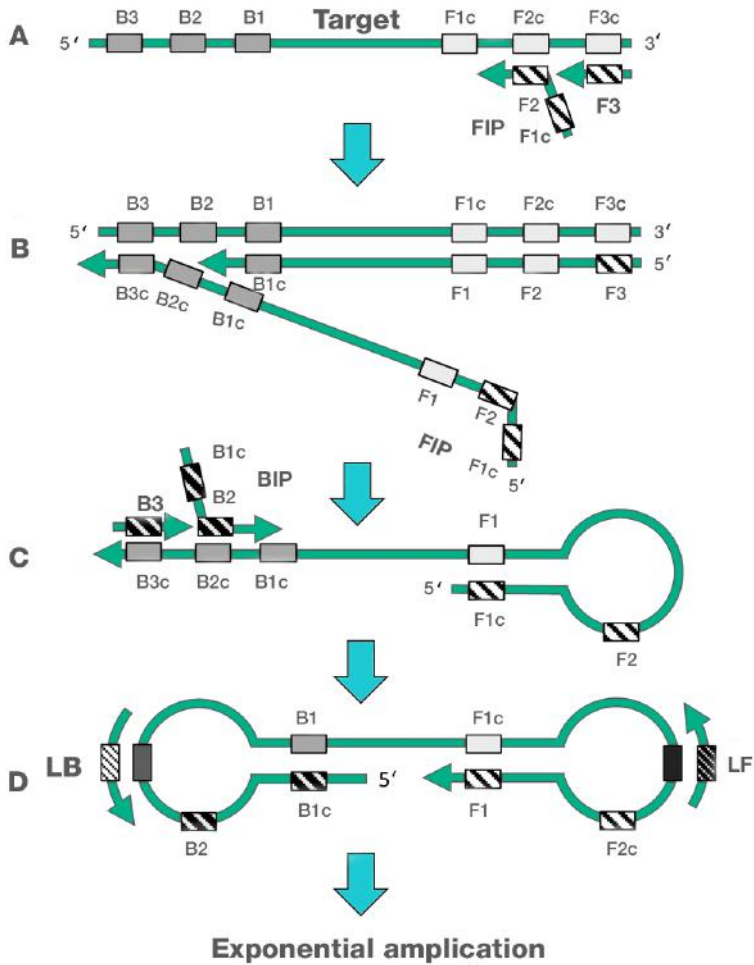


WHAT IS LOOP-MEDIATED ISOTHERMAL AMPLIFICATION?

Loop-Mediated Isothermal Amplification (LAMP) is a Nucleic Acid Amplification Technique (NAAT) invented in the late 1990's by the Japanese Eiken Chemical Company. LAMP amplifies DNA at a constant temperature between 60 and 65°C.

LAMP utilises a *Bacillus stearothermophilus* derived polymerase that has strand displacement activity and a set of four to six sequence specific primers to generate large, concatenated double-stranded stem and loop amplification products. LAMP can also be coupled with a compatible reverse transcriptase (RT) to enable the real-time detection of RNA.





Adapted from Von Stetten et al. Anal. Methods, 2020, 12, 717-746
20, 12, 717-746

LAMP uses four to six sequence specific primers for between six and eight distinct regions of target DNA. A strand displacing *Bacillus stearothermophilus* DNA polymerase initiates the synthesis and two of the primers form loop structures to facilitate further rounds of amplification.

THE ADVANTAGES OF LAMP

LAMP is considered to have several advantages over many of the other isothermal amplification techniques. These include:

Speed and sensitivity: LAMP produces a large amount of amplified product in a relatively short period of time providing good assay sensitivity with up to 10^9 -fold amplification generated in less than an hour from a single copy of DNA.

Specificity: LAMP is also highly specific, employing four to six sequence specific primers compared with two to three typically used in other amplification techniques.

Robustness: LAMP has been demonstrated to have good tolerance to inhibitory substances compared to many other amplification technologies, making it robust to carryover of potential inhibitors in the sample matrix. This allows for use with less-refined sample types, simplifying workflows and enabling field-based applications.

Versatility and utility: LAMP is compatible with multiple detection technologies allowing for utilisation of assay formats including colorimetric, turbidimetric, lateral-flow, fluorescence and electrochemical. Often the instrumentation requirements for these formats have lower technical complexity and power requirements. This enables the cost-effective mass-manufacture of portable, battery powered devices that can be operated in challenging geographies, environments and near-patient settings.

Accessibility: since the expiry of the original patents and licence agreements that previously limited the commercial use of LAMP, there has been an explosion of its applications for *in vitro* diagnostics. There are also an increasing number of commercial vendors that supply reagent components and complete assay reagents compatible with LAMP.

THE CHALLENGES OF LAMP

As with all amplification technologies there are several challenges to consider and overcome when developing LAMP assays.

Primer design: LAMP primer design is more complex than PCR because LAMP requires four to six primers, usually double the number for most other isothermal amplification techniques. This makes designing compatible primers more challenging, particularly for small target sequences.

Potential for false positives: in some LAMP assays an observed false positive phenomenon can occur towards the end of assay duration. This is thought to be due to the high efficiency of amplification and the number of primers involved. This can be nullified by utilising the correct primer design and through the addition of chemical components to the assay formulation.

Stabilising assay reagents for challenging conditions: stabilising dried assay reagents to have the required shelf-life at temperatures above ambient requires specific expertise. Selecting the correct stabilising formulation and 'drying' process is critical.



HOW WE CAN HELP WITH YOUR LAMP ASSAY REAGENT DEVELOPMENT AND MANUFACTURING

We have extensive experience aiding our customers develop LAMP assays, having worked on more than 20 LAMP projects since 2020, and many more prior to that.

We understand many of the challenges, having co-developed assays utilising a wide array of detection platform technologies and reagent deposition processes, including lyo bead format and drying onto a range of porous and solid material substrates compatible with lateral-flow technology.

Supported by our in-house molecular biologists and purpose-built laboratories and manufacturing facilities, we can help you with everything from your LAMP primer design, assay development, reagent stabilisation, shelf-life determination and manufacturing scale up to commercial volumes.

To find out more about how we can help you develop your next diagnostic assay, please get in touch using the contact details on the back page.



GET EXPERT TECHNICAL ADVICE

Biofortuna is a specialist contract development and manufacturing organisation providing genomic services, custom development and manufacturing support to the *in vitro* diagnostic, consumer health, life sciences and pharma biotech sectors.

As an FDA registered, ISO 13485 and ISO 17025 accredited provider, we integrate our quality management system and design control standards within all our assay design, development and manufacturing projects.

Contact us today for an informal discussion around how we can help you bring your assays to market faster.

E&OE excepted: 02/25



+44 151 334 0182
www.biofortuna.com

Biofortuna Limited
2 Tenth Avenue
Deeside Industrial Park
Deeside. CH5 2UA



Winner of the Technical Service Award for our Lyo bead development and manufacturing service



Shortlisted for the Internationalisation Award in recognition of our export growth

