Pathogen Detection: Loop-mediated Isothermal Amplification (LAMP)

Pathogens continue to be a real threat to public health and to food and beverage processors' businesses. Time and again, contaminated food causes illnesses many of which turn out to be fatal. Another effect is a considerable economic loss. A faster, easier, and less expensive test for pathogen detection is clearly a need for the industry.

In 2011, a survey performed by Food Control & Inspection on about 485,000 food processing operations revealed 150,000 objections. That amounts to more than a quarter of all operations in the survey. When presenting their annual Food Control & Inspection Report, BVL, the Federal Office for Consumer Protection and Foodstuff Safety, attributed most of the objections to general industrial hygiene. 53 % of the companies failed the inspection in that respect. Nearly a quarter were reprimanded for their hygiene policy. Almost 52,500 (...) of the about 402,000 food samples gave rise to complaints in 2011. Whereas just under 51 % of the samples were reprimanded for their labeling and appearance, microbiological contaminations were found in nearly 18 % [1].

Through an extensive collaboration process with global customers and industry, 3M developed a novel approach, utilizing molecular microbiology for pathogen detection.

The molecular detection system works with color-coded assay tubes and is based on a combination of technologies - loop-mediated isothermal amplification (LAMP) of DNA and bioluminescence detection. The innovative combination of these technologies makes the system a cost-effective, easy-to-use method with the molecular accuracy demanded by customers.

Loop-mediated Isothermal Amplification

The chemistry in the detection assays is predicated on LAMP, which is the result of novel developments in molecular biology supporting widespread deployment for rapid molecular testing. Scientific literature recognizes it as a highly robust, efficient, sensitive, specific, and simple nucleic acid amplification technique. This
translates to faster time-to-result and reduced complexity and cost as compared to Polymerase Chain Reaction (PCR) methods. Studies show that when compared to PCR, LAMP has higher sensitivity and greater ability to amplify DNA in the presence of interfering substances often found in food and environmental samples.

LAMP technology allows the molecular detection system to offer a simple sample preparation process with only two transfer steps, instead of the more complex DNA extraction and purification steps typically used in PCR.

It also utilizes multiple primers that recognize distinct regions of the target gene.

The reaction takes place at a constant temperature, unlike PCR, which requires time-consuming thermal cycling. The method continuously amplifies the target DNA, resulting in a process that is both highly specific and rapid, generating more than one billion copies of the target in as little as 15 minutes.

Robust Bst DNA Polymerase
Bst polymerase is a unique enzyme with DNA strand-displacement activity which enables the continuous, rapid isothermal amplification of LAMP, unlike PCR in which DNA extension is limited to a specific period of time within the thermal cycling process. In PCR, the presence of inhibitors can prevent the polymerase from extending the DNA in the time allowed, producing incomplete amplification products and preventing the detection of the target organism [2].

According to Nkouawa et al., "due to the tolerance of the Bst DNA polymerase to inhibitors, in contrast to the Taq DNA polymerase (used in PCR), LAMP appears as a gold standard method to detect pathogens in ... specimens that are known to contain a large amount of inhibitors." [3]

Bioluminescence Detection
The unique method of bioluminescence detection, combined with the single-
temperature amplification of LAMP used by the molecular detection system, eliminates the need for the large and complex instrumentation required in PCR-based systems (fig. 1).

The need for internal controls in PCR evolved as a result of PCR's limitations and susceptibility to inhibition from sample matrices [4], which can lead to false negative results.

The molecular detection assay system utilizes the combination of LAMP and bioluminescence detection which results in a simpler, more robust DNA amplification and detection method providing significant user benefits over common PCR technology. Unlike PCR methods, the reliable technology used in the molecular detection assay does not require an internal amplification control in each tube. Internal amplification controls add cost, complexity, and have been shown to reduce assay sensitivity [5].

It is important to recognize that inhibition of DNA amplification is not the only cause of false-negative results in PCR. PCR methods can involve reagent preparation and complicated sample processing steps, which are typically uncontrolled. The molecular detection system provides ready-to-use reagents and a simple protocol, eliminating many of the uncontrolled steps involved in PCR.

The molecular detection system uses bioluminescence technology to report the DNA amplification of the target organism in real-time. This involves a two-step enzymatic process in which pyrophosphate molecules, produced as a byproduct of the DNA amplification, are used to generate light. This light emission is then easily read by the molecular detection instrument and signals the detection of the target organism.

The bioluminescence detection is robust, reliable, and resistant to sample interference. In contrast, fluorescence detection, which is used in many PCR-based systems, can be subject to interference by the natural fluorescence of some food samples and enrichment media.

**Summary**
The unique method of bioluminescence detection, combined with the single-temperature amplification of LAMP used by the molecular detection system, eliminates the need for the large and complex instrumentation required in PCR-based systems. The system is proving valuable as companies put it to the test on their food matrices.

More remains to be learned, but this much is clear: simplicity, efficiency, and intuitiveness are paramount in developing sustainable solutions that consistently
protect brands and the reputations of companies to help bring quality and safety to the table.

References

Authors
Mathias Boldt, Scientific Marketing & Technical Service Manager, 3M Food Safety

Contact

3M Deutschland GmbH
Carl-Schurz-Str. 1
41460 Neuss
Germany
Phone: +49 2131 14 0
Telefax: +49 2131 14 2649