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Technical Bulletin

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3M™ Molecular Detection System
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The 3M™ Molecular Detection System—Enabling Control for Food-borne Pathogen Detection

The 3M™ Molecular Detection Assay 2 collection is the next generation of assays for the 3M™ Molecular Detection System. The method is based on a unique combination of technologies – loop-mediated isothermal DNA amplification (LAMP)[1] and bioluminescence detection. Combining these technologies, the 3M Molecular Detection Assay 2 platform makes molecular detection of foodborne pathogens simpler and faster, providing food manufacturers never-before-available speed and ease in simultaneously identifying *Salmonella*, *Listeria*, *Listeria monocytogenes*, *E. coli* O157 (including H7), *Campylobacter*, and *Cronobacter* in food and/or environmental samples.

LAMP is recognized throughout the scientific literature as a highly robust, efficient, sensitive, specific, and simple nucleic acid amplification technique[2,3]. LAMP uses strand-displacing *Bst* DNA polymerase and 4 to 6 primers to produce continuous DNA amplification at a single temperature. In contrast, Polymerase Chain Reaction (PCR) uses a non-strand displacing *Taq* DNA polymerase and two primers. *Bst* polymerase used in LAMP is more robust and less prone to inhibition than *Taq* Polymerase[4-8].

In PCR, DNA extension is limited to a specific period of each thermocycle. 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[9]. PCR's temperature cycling and the association and disassociation of the polymerase from the DNA template during the denaturation step, provides many opportunities for inhibitors to interfere, compared to LAMP which is a single-temperature, continuous amplification without dissociation of polymerase from DNA template.

Bioluminescence detection is robust, reliable, and resistant to sample interference[10]. In contrast, fluorescence detection, which is used in many PCR- and Immunoassay-based systems, can be subject to interference by the natural fluorescence of some food samples and enrichment media[11].

What controls accompany the 3M Molecular Detection System?

The 3M Molecular Detection Assays and 3M Molecular Detection System utilize a unique and proven combination of technologies, packaging, design, and customer support to provide the end user with optimally controlled results without relying on an Internal Amplification Control (IAC). The 3M™ Molecular Detection Matrix Control is an external control that serves the same purpose as an IAC to

measure matrix interference with LAMP and bioluminescence. Assurance that amplification reagents are active is provided during each run with the 3M™ Molecular Detection Reagent Controls provided with each assay kit.

Most importantly, 3M Food Safety is committed to your success and can assist you with microbiological method validation studies to not only demonstrate a lack of matrix interference, but also verify that 3M's recommended enrichment protocols will in fact grow low levels of target pathogen in your unique food and environmental samples.

It is important to recognize that inhibition of DNA amplification, as evaluated by amplification controls (IACs or external controls), is not the only root cause of incorrect risk management decisions. Amplification controls do not ensure the entire sample testing process runs perfectly. The amplification controls do not evaluate whether 1-5 CFU of a target pathogen was able to grow to the detection limit under the enrichment conditions (dilution, buffer, time, temp) or if bacterial cells were completely lysed in any given sample.

From the early stages of food safety planning through sample test confirmation, many factors influence whether or not your desired risk management action is taken to protect your brand. Accurate pathogen testing depends upon a system of scientifically validated and well executed prerequisite programs, preventative controls, sampling plans, sample collection, sample preparation and enrichment, sample testing, sample test confirmation, and risk management procedures. 3M Food Safety support, education, and consultation assists your efforts to control some of these critical factors outside of the laboratory, while the 3M Molecular Detection System delivers a reliable and proven method to assess accurate pathogen testing results, on time, the first time.

A Systems Approach to Accurate Microbiological Hazard Risk Management

Prerequisite Programs	Preventative Controls	Sampling Plans	Sample Collection	Sample Prep/Enrichment	Sample Testing	Test Confirmation	Risk Management
•Appropriate •Validated •Executed	•Appropriate •Validated •Executed	•Appropriate •Validated •Executed	•Appropriate •Validated •Executed	•Validated •Executed	•Validated •Executed	•Appropriate •Executed	•Aligns with risk tolerance

Why do PCR systems utilize an IAC?

- It is well established that PCR is inhibited by various compounds found in foods and environmental samples[9, 12, 13].
- PCR assay kits are laborious and confusing to prepare correctly, with several buffers that need to be hydrated, aliquoted, and stored under specific conditions. An internal control is required to prove all reagents were prepared, aliquoted and stored correctly.
- PCR systems have no software control to indicate when the amplification conditions fail to provide the necessary temperature and cycles, or when amplification/detection reagents are missing from reaction wells.
- PCR systems utilize an enzymatic lysis buffer containing a protease enzyme that will inhibit DNA Polymerase amplification if the protease-based lysis buffer is not entirely inactivated during lysis heating.
- PCR systems utilize varying lysis buffers depending on the pathogen assay and protocol. The use of the incorrect buffer can inhibit a PCR reaction, resulting in a false negative.

What are the drawbacks of amplification controls for molecular pathogen detection?

- Amplification controls only measure inhibition of DNA amplification by possible inhibitors in food sample or lysis buffer.
- IAC DNA templates compete for amplification and detection reagents such as DNA polymerase, free nucleotides, and magnesium in each reaction, and reducing assay sensitivity[12, 13].
- IAC inhibition does not always equal target amplification inhibition. Valid IACs do not always indicate a successful target detection would have occurred.
- ISO 22174:2005 recommends use of positive process controls (samples spiked with target pathogen and processed in the same way) and not just internal OR external controls[14].

What are some potential pathogen testing failures that can arise from reliance on PCR IACs?

- Reliance on IACs may lead to neglect of microbiological method verification studies as recommended by FDA, USDA, and ISO 16140[15]. These studies, utilizing live culture controls, serve to verify not only the amplification and detection reactions, but also sample collection, preparation, and enrichment processes.
- It is possible that excess sanitizer, food compounds, or background flora in samples may slow or inhibit the growth of the target pathogen during enrichment. This is not evaluated by IACs. It is possible that a sample containing the target pathogen can be ruled negative by a PCR system, while the IAC is ruled valid, resulting in a truly "false negative" result.

Why does the 3M Molecular Detection System not utilize IACs?

- LAMP technology is not inhibited by known PCR inhibitors or by sanitizers utilized in the food industry. For example, in a variety of liquid pasteurized egg matrices[16] and spices[17, 18] showing inhibition by some common PCR-based rapid screening methods, LAMP-Bioluminescence technology in the 3M Molecular Detection System did not show any inhibition of amplification. Peer-reviewed studies show the 3M Molecular Detection System performs well with a wide variety of foods[19-35].
- 3M Molecular Detection Assays are 100% racked and ready-to-go. There are no buffers or reagent mixes that must be prepared, aliquoted, or stored. There is no risk of a false negative due to incorrect preparation of the lysis buffer or amplification/detection reagent.
- The 3M Molecular Detection System utilizes a single lysis buffer for all assays and protocols. It is not possible to get a false negative result due to use of incorrect lysis buffer.
- The 3M Molecular Detection System contains software controls to indicate when amplification conditions were not met by the instrument, allowing users to make risk management decisions based upon their unique food safety plans and already determined real-time results.
- The 3M Molecular Detection Assay 2 family utilizes a chemical lysis buffer instead of an enzymatic lysis buffer reducing the possibility of incomplete lysis and not requiring use of amplification controls in every reaction.
- The 3M Molecular Detection System contains a software control that will rule any assay well without the assay pellet as an "error" within the first 60 seconds of an assay run.

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