

# Rapid, accurate pathogen testing systems

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The science and engineering behind the development of new systems for detecting pathogens continues apace. There are always the challenges of accuracy of detection, the time it takes to get a meaningful result, the constraints of staff knowledge with limited expertise and the cost.

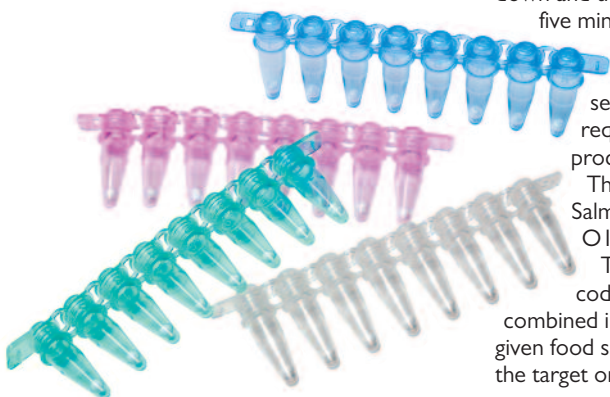
The advent of a new system will always attract attention from all who either have responsibility for the process of carrying out the test or from those who are reliant on the results.

The industry is long familiar with the rapid hygiene test systems based on ATP bioluminescence, but it has been used as a broad indicator of cleanliness rather than signalling precise bacterial contamination.

More recently the food industry has benefited from the development of PCR testing based on the replication of target genetic material to be measured as a highly accurate indicator of specific microbial contamination. Each method has its own benefits and disadvantages. ATP is fast but not organism specific, whilst PCR has tended to trade complexity and cost for precise identification. Each method has been harnessed into their respective testing systems to which the old saying of 'horses for courses' can be truly applied.

If only there was a system that was as fast and simple as ATP and as precise as PCR.

**Colour coded reagent tubes enable controlled parallel testing.**



The developers of new ideas at 3M have an unrivalled reputation for meeting such challenges. Following three years of research and development the company has launched the 3M Molecular Detection System. It is said to offer the combination of simplicity, speed and accuracy.

So how does it work? How can it take an enriched food sample and provide a precise contaminated/not contaminated result in less than two hours.

What 3M scientists have done is to develop a simplified and rapid isothermal DNA amplification step to quickly multiply the targeted DNA and then applied the principles of bioluminescence detection to identify its presence. The system is based on a molecular detection instrument that is very compact and simple to use and is connected to a standard laptop.

The laboratory technician would start by switching on both the laptop/software and the instrument which then warms up for 20 minutes. The technician places the required number of 20µL of enriched sample in lysis tubes. The tubes are then heated at 100°C (± 1°C) for 15 minutes to break open the cells and release the DNA.

Then the tubes are placed into a chill block for 10 minutes to cool down and then removed to sit for five minutes to reach room temperature.

The technician has meanwhile selected the reagent tubes required and placed them into the processing tray.

The choice at the moment is Salmonella sp, Listeria sp, and E. coli O157 (including H7).

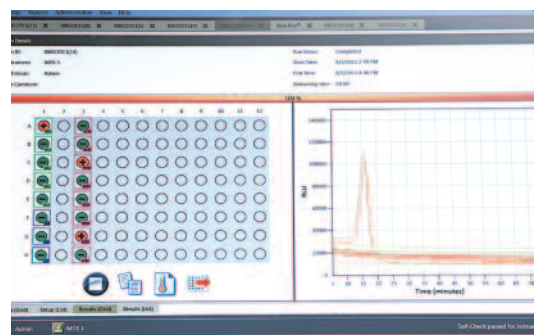
The reagent tubes are colour coded and can be selected and combined in the processing tray so that a given food sample can be tested for each of the target organisms in the same run.



**3M Molecular Detection System.**

The program is then selected on the laptop either using a previously set test pattern or by creating settings tailored for a new customer/project. The software will use this data to monitor, measure and report the results.

The capped assay tubes each have a self contained reagent pellet to which is added the 20µL of lysate. The tubes are transferred into a speed loader tray and this is put into the instrument which then processes the tests. A positive result will



**3M Molecular Detection Software.**

usually be readable as early as 15 minutes, but the system runs for 75 minutes to confirm the negative results.

How does it actually work? The assay moves through two stages. The first stage, isothermal DNA amplification, recognises the distinct region of the genome for the target organism and the DNA polymerase initiates strand displacement of the targeted organism and efficient, rapid and continuous multiplication of the target DNA.

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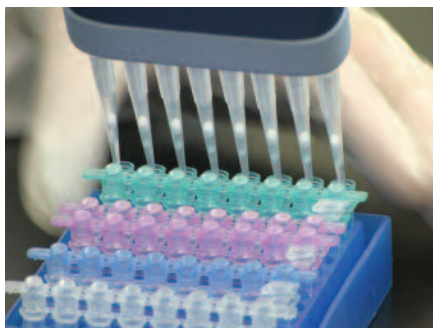
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A by-product of the amplification is the release of pyrophosphate.

The ATP sulphurylase in the reagent mix then converts this by-product to ATP and the third element of the mix, luciferase, then triggers the production of light to be measured. Amplification and detection occur simultaneously and continuously during the exponential phase providing real-time results and the short run time.

In a positive sample it usually takes about 15-20 minutes for the light output to reach a detectable level and be measured. The reading is stored and presented on the lap top. Each run of tests is monitored by the inclusion of a negative control test and a reagent control test to verify that the test

#### **Dispensing sample lysate into colour coded reagent tubes.**



#### **Assays for *Salmonella* sp., *Listeria* sp. and *E. coli* O157 (including H7).**

process has been completed correctly. The molecular detection system is not only quicker to get a result, but it also supports the trend in laboratory staffing toward the increased use of competent technicians rather than highly qualified scientists for regular day to day testing procedures.

This is achieved by keeping the processes straightforward and simple to follow, with the same protocol for each pathogen.

The instrument and software combination then ensures the accurate and reliable interpretation of results. The equipment is sturdy and designed to be easy to use, thereby avoiding the need for high capital investment in complex multi-process systems.

The use of the isothermal DNA amplification method reduces the likelihood of the complications of amplification systems that use temperature cycling.

The use of the 'all-in' ready to use pellet system for carrying the active agents means that there is no need to make up reagents or follow a more complicated multistage process that needs more time and offers the opportunity for error or contamination.

The instrument has no moving parts, requiring no cooling fans and does not use expensive thermocyclers, fluorescence detectors or filters.

It therefore does not require re-calibration each time it is redeployed and maintenance is minimal.

The small footprint means that it takes up little room on the workbench, and in the event of a spill within the test cabinet it is very easy to clean and decontaminate.

The system has been trialled using a wide range of food matrices and it is relatively straightforward to carry out inhibition trials to benchmark a new food matrix.

The low investment threshold, modest size and ease of operation mean that the molecular detection system is able to provide a rapid and accurate test solution for modest laboratory operations.

With 94 test cells available and the ability to complete perhaps four cycles in a regular working day it could be possible to test as many as 120 samples a day for the three targeted organisms. For higher throughput the software can operate up to four instruments simultaneously so capacity can be expanded for a relatively modest outlay. ■