

Microbial update

Listeria

Produced as a service to the food industry by Oxoid Ltd.

A recent recall of sandwiches supplied to a variety of establishments in southeast England has once again highlighted the potential threat to food safety posed by products contaminated with listeria.

In this instance, the presence of the pathogen was picked up by workers in a local authority laboratory as a result of routine sampling.

The discovery led to immediate suspension of production in the factory involved.

Of the six recognised species of listeria, only one – *Listeria monocytogenes* – is generally associated with disease in humans. However, members of the genus listeria are known to co-exist in mixed populations and in similar environmental conditions.

Therefore, detection of any of the species in a sample should be enough to raise suspicion that *L. monocytogenes* may also be present.

Listeria are short, Gram positive bacilli which are motile at 20-25°C, non-motile at 37°C and characterised by being facultatively anaerobic, catalase positive and oxidase negative. In addition, *L. monocytogenes* is β -haemolytic on blood agar, a fact closely linked with its pathogenicity.

Being ubiquitous in the environment, it is possible for listeria to be present in most food produce or to contaminate food during processing. In addition, listeria have the ability to survive the manufacturing and ripening of many different cheeses and can often be found in cottage cheese and other mildly acidic dairy products. It may also be isolated from raw and pasteurised milk, cream, butter and ice cream – an area of particular risk where children are con-

Ice cream can be an area of concern for children (Shutterstock).



cerned. Growth of *L. monocytogenes* on meat and meat products is highly dependent on product type and pH.

The organism tends not to grow well on meat products that have a pH value near to or below 5.0. Pâté on the other hand, has been shown to be capable of growing *L. monocytogenes* in high numbers.

Listeria are also relatively resistant to meat curing processes and have been isolated from salami and vacuum packed ham products.

Chicken can also be heavily contaminated with listeria, with different surveys showing contamination rates ranging from 12-60%.

The pathogen has also been found to survive well in many other food types including egg, potato, salad and sea foods such as smoked salmon and shrimps.

A particular headache for food manufacturers results from the fact that listeria can grow at low temperatures where most competing organisms would find growth difficult.

This means that refrigeration may actually selectively encourage growth of the organism.

Cook-chill, ready-to-eat meals are of particular concern considering the pathogens ability to survive low temperatures. Special care must, therefore, be taken to try and exclude it completely from food products and the production environment wherever possible.

Serious health risks

In healthy individuals, infection with *L. monocytogenes* can often be asymptomatic.

However, vulnerable groups such as pregnant women, young babies, the elderly and those with lowered immunity are at risk of developing listeriosis – a disease which can prove to be life threatening. The symptoms of listeria infection (fever, flu-like illness and/or diarrhoea) may take up to 90 days to develop. When associated with infection



Pâté has been shown to be capable of growing *L. monocytogenes* in high numbers (Shutterstock).

of the foetus in pregnant women, listeriosis can lead to abortion, stillbirth and premature delivery.

Preventative measures

In attempting to minimise the possibility of listeria being present in their products and processes, food manufacturers must not only take steps to monitor raw materials and product, but environmental samples as well. Drainage systems, guttering, floors and other surfaces may often be found to be harbouring listeria. Hazard Analysis Critical Control Point (HACCP) procedures must, therefore, be implemented in the manufacturing process to reduce the potential for contamination. Failure in these procedures can have worrying implications from a consumer safety perspective, not to mention the commercial damage of stopping production or recalling a product due to the presence of listeria in a final product.

In an ideal world, listeria would be completely absent from all ready-to-eat food products at the time they are released to the consumer. In the real world, however, appropriate microbiological criteria need to be set for the guidance of food manufacturers.

Such criteria can be found under EC Regulation 2073/2005 which came into force in January 2006, providing important safeguards for both manufacturers and consumers.

The General Guidance document issued by the UK Food Standards Agency with respect to this Regulation provides some

useful guidelines for determining whether *L. monocytogenes* would grow in a particular product, and ways in which businesses may demonstrate compliance with the microbiological criteria set.

Manufacturers are advised to consider whether the physico-chemical properties (for example, pH, salt levels and water activity (*a_w*)) of a particular product would prevent the growth of *L. monocytogenes* during storage, and whether it would be possible to control growth by modifying, for example, the water activity or the level of preservatives used.

Different microbiological criteria are set in this guidance document, depending on the nature of the food. For example, where ready-to-eat foods are intended for infants or special medical purposes, *L. monocytogenes* should be absent in a 25g sample throughout the product's shelf life.

Where the shelf life is five days or less, or the foodstuff is unable to support the growth of the pathogen, *L. monocytogenes* should not be present at a level exceeding 100 colony forming units per gram (cfu/g) during the shelf life period.

The same criterion also applies to products whose shelf life has been set taking growth of *L. monocytogenes* into account. In this instance, if shelf life studies show that 100cfu/g is likely to be exceeded before the end of the shelf life, consideration must be given to reviewing the shelf life, reviewing food safety management procedures or ensuring that the pathogen is absent in a 25g sample before the food leaves the production facility.

This latter case would typically be achieved by operating a positive release system, which can in turn have an impact on the supply chain and available shelf life of the product.

Detection methods

The methods used for detecting and enumerating listeria vary between laboratories, depending on the country in which they are situated, the type of sample being tested (food or environmental), and individual manufacturers' specific needs. Some laboratories will look for *L. monocytogenes* specifically, while others will seek to detect the genus, recognising the propensity of different species to co-exist.

Where presence/absence answers are required, it is first necessary to enrich any listeria organisms present in order to facilitate subsequent detection.

The widely adopted ISO method for the detection of *Listeria monocytogenes* (ISO 11290-1 incorporating amendment 1), utilises Half Fraser Broth as the enrichment medium, followed by plating onto listeria agar according to Ottaviani and Agosti (ALOA) and a second medium of choice such as PALCAM Agar. ALOA is a chromogenic medium which detects β -glucosidase activity, causing listeria colonies to



The BAX system Q7 from Qualicon enables quick detection of listeria.

appear blue. *L. monocytogenes* and pathogenic *L. ivanovii* can be further differentiated from other listeria species by the presence of an opaque halo around the colonies.

This is due to the action of phosphatidylinositol phospholipase C (PIPLC) – produced by pathogenic species – on phosphatidylinositol present in the medium.

Recently, OCLA ISO, a novel and cost effective alternative to this medium was launched by Oxoid which incorporates the less expensive lecithin to detect phosphatidylcholine phospholipase C (PCPLC), also associated with pathogenicity.

This current ISO method represents a considerable improvement over its predecessor in terms of the clarity of results produced by the chromogenic agar.

However, the time to a confirmed negative result of up to five days remains a disadvantage as far as food manufacturers are concerned. The dilemma when testing short shelf life products is whether to operate a positive release system (thus effectively reducing their post-distribution life) or to release them prior to obtaining test results (in which case a highly efficient monitoring and recall system is essential).

Either way, speed of testing is very much of the essence, and accuracy is vital. So, too, is the need for results to be both unequivocal and easy to interpret. The commercial consequences of retaining or recalling a product on the basis of a false positive result can be disastrous.

In an effort to resolve this dilemma, various alternative rapid methods have been developed to speed up the process of testing for listeria whilst enhancing ease of use and accuracy. These include rapid culture methods, enzyme-linked immuno-sorbent assays (ELISAs) and lateral flow tests such as Oxoid's Listeria Rapid Test.

Typically, these methods can provide presumptive identification of listeria species from culture 24-48 hours earlier than conventional techniques. When using such tests, however, it is important to remember that results still need to be confirmed by means of biochemical analysis.

Advances in molecular techniques have also helped make the process of detecting and identifying listeria quicker and more convenient.

The use of automated DNA-based polymerase chain reaction (PCR) systems

enables detection of listeria from samples following enrichment in a matter of hours rather than days.

Availability of PCR systems such as the BAX System Q7 from Qualicon is helping to overcome the negative perceptions which have traditionally made laboratories reluctant to adopt PCR technology.

This reluctance is rooted in the widespread belief that PCR systems are expensive, that they require highly skilled personnel to operate them and that they need to be used in a separate room in order to avoid the risk of DNA amplicon contamination. Such preconceptions are overcome by the BAX Q7 methodology, which combines all the necessary PCR reagents for listeria testing in a single tablet, conveniently packaged inside the PCR tubes.

Use of tableted reagents minimises hands on time whilst at the same time enhancing accuracy, shelf life and consistency. Closed cap processing ensures that amplicon contamination is a thing of the past.

Validation studies have shown that this system performs as well as traditional culture methods for detecting *L. monocytogenes* in food with 98% sensitivity/specificity.

What is more, this, and many other PCR systems have been approved by international bodies such as the AOAC and AFNOR. As a result, use of PCR technology is now increasingly being seen as a viable proposition by more and more food industry laboratories. Because of the importance of *L. monocytogenes* in foodborne infections and the widespread distribution of listeria species in the environment, the importance of rapid detection in the food industry cannot be underestimated.

Recent incidents such as the recall of sandwiches in southern England should serve as a salutary warning to us all.

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