Australian food company compares listeria testing options

by Philip Mugg and Alan Deery, Microgen Bioproducts Ltd, Camberley, Surrey GU15 3DT, UK.

To ensure products are free from microbial contamination food manufacturers are increasingly adopting environmental sampling programs as part of their HACCP (Hazard Analysis Critical Control Point) plans.

An environmental monitoring programme focused on risk assessment enables the detection of microbial contamination, particularly the detection of important food pathogens in a timely manner. If samples are taken in a planned manner to reflect the differing working conditions and the results are tabulated correctly, meaningful comparisons and the analysis of trends can be examined easily.

The importance of microbial contamination and the implications for consumer safety and commercial damage is highlighted by an outbreak of listeriosis caused by contaminated meat products in Canada.

The outbreak was linked to 22 deaths and cost the company $20 million in a product recall of 220 products and $25-27 million to settle law suits.

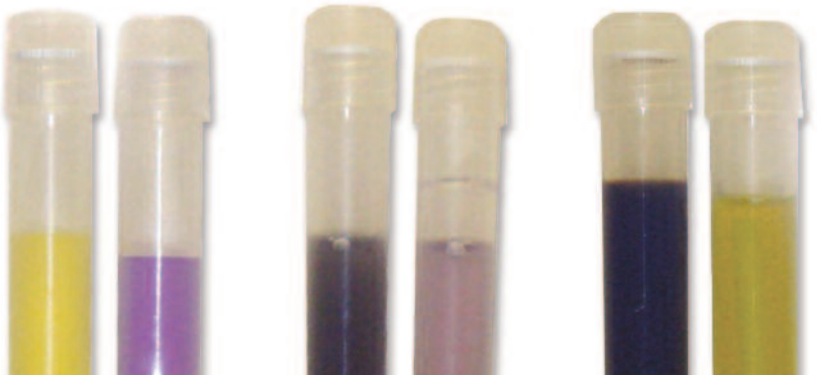
Widespread problem

Listeriosis is caused by Listeria monocytogenes, the pathogenic species of the genus listeria, a Gram positive, catalase positive and oxidase negative group of organisms.

The disease is a serious problem as it has a high fatality rate (>25%). Listeria is widespread in the environment so consequently there is considerable opportunity for food products and food handling environments to be contaminated.

The problem of listeria contaminating food is a concern for any food manufacturer as the organism grows well in a wide range of salt concentrations, pH's and temperature conditions giving it a competitive advantage over other mesophilic flora.

A variety of methods can be employed for environmental monitoring such as visual analysis, ATP detection and the detection of surface protein residues as well as pathogen specific environmental monitoring.



Path-Chek Hygiene Pathogen detection broth. Positive reactions are on the left and negative are on the right.

However, at the moment these methods do not demonstrate the presence of specific food poisoning bacteria. They either detect the presence of bacteria non-specifically, or food residues on surfaces that will most likely harbour bacteria. Only specifically targeted environmental testing methods can detect the presence of specific food pathogens capable of causing food poisoning or worse, present in the environment, which may not have been eliminated by routine cleaning and sanitising procedures.

The detection of adenosine trisphosphate (ATP) is an established method of hygiene monitoring within the food industry. ATP analysis is the detection of a nucleotide which exists in all cells so it does not specifically detect pathogens but acts as a surrogate marker of contamination. ATP analysis is not a microbiological method in that it does not target ATP specifically from bacteria.

ATP tests provide a rapid result but it does not indicate if the ATP detected is from bacteria and whether the bacteria are important pathogens so it can be used as a complementary tool to rapid and effective pathogen monitoring. Detection of specific pathogens within the manufacturing and/or processing environment is vital to detect the presence of important food pathogens introduced into the food handling environment and highlight the sources of these pathogens which may be resident in the environment.

Traditionally food manufacturers either have to send environmental samples to commercial laboratories for analysis, which can be expensive and timely, or follow traditional microbiological methods of environmental monitoring. The traditional environmental microbiological testing methods for listeria involves sample swabs being incubated for 48 hours in an appropriate broth medium followed by subculture onto a suitable agar plate medium such as ALOA and/or Oxford agar. This is a laborious process which can take up to five days to achieve a final result. The time to result and the three step process is a disadvantage for any food manufacturer especially if they operate a positive release programme and this is limited to those companies large enough to have suitable laboratory facilities or access to contract testing laboratories.

Pathogen detection system

Microgen Bioproducts has overcome problems associated with these traditional methods of environmental monitoring by the introduction of their Path-Chek Hygiene Pathogen system for the detection of important foodborne pathogens (Listeria spp, coliforms and Salmonella spp.) from work surfaces and manufacturing equipment in food handling and manufacturing environments. The pathogen detection system consists of two units; a pre-moistened swab, which has the benefit of neutralising the effects of cleaning solutions and improving bacteria recovery from dry surfaces; and a

Continued on page 23

Continued from page 21

highly specific and sensitive detection media which gives results by providing a clear visual colour change in 18-24 hours for coliforms and Salmonella spp. and 30-48 hours for Listeria spp. if specific organisms are present on surfaces.

The pathogen detection system meets the requirements of ISO:18395:2004(E) and is compliant with the requirements of USDA, FSIS and BAM but unlike similar methods does not require a pre-enrichment step.

One of Australia's largest food manufacturing companies (Dairy Farmers) evaluated Path-Chek Hygiene Listeria to determine its effectiveness in the isolation of listeria from environmental samples. The work was carried out by David Wong, scientific services manager at Dairy Farmers, Australia.

His investigation included parallel testing using a well known automated immunoanalyser from a NATA accredited laboratory (NATA is Australia's internationally recognised laboratory accreditation authority) as well as an internationally marketed media based system.

Environmental swabs were collected within one of the Dairy Farmers manufacturing sites. In each area, three separate swabs were taken within a five minute period to minimise variability and each site was sampled five times over a five week period. One swab from each site was tested for the presence of Listeria spp. using each of the systems under evaluation.

The listeria targeted media based system is a quantitative method which involves sample ready plates, which are inoculated and incubated for 29±2 hours after an environmental sample has been resuscitated.

The immuno-analyser system is an automated qualitative instrument which utilises ELFA (Enzyme Linked Fluorescent Assay) technology. Environmental listeria samples for analysis in this system require pre-enrichment in half Fraser broth for 24 hours followed by enrichment in Fraser broth for 24 hours.

After a sample has been pre-enriched and enriched, 0.5ml of the Fraser broth inoculates the test strip, which is then analysed automatically by the instrument and a test value generated for each sample.

In this study the culture media film demonstrated a lower sensitivity, with detection of only 47% of the confirmed positive samples, in comparison to Path-Chek Listeria (100%) and the immuno-analyser (87%).

Path-Chek Listeria exhibited a false positive rate of 12% based on initial visual interpretation, followed by confirmation by subculture and the identification of suspect colonies in their laboratories.

These false positive results are, however, still considered valuable as they highlighted high background levels of certain organisms such as Bacillus spp. and Enterococcus spp., which are good indicators of a poor hygiene level or poor sanitising practices. A high false negative rate is not acceptable as it may result in the release of food products with high bacterial loads which may result in rapid food spoilage.

The lack of sensitivity of the media based system is possibly due to the fact that this method does not involve an enrichment step. Such a step would increase the overall sensitivity, particularly when a low number of cells are involved or cells have been damaged or stressed by temperature, detergents or sanitisers.

Path-Chek and the media system are both low cost in-house methods used for environmental monitoring ,although the latter requires the expertise of a microbiologist as there are two aseptic steps (resuscitation and inoculation). It is a simple one-step closed system as the swab tip is snapped off into the detection tube, ensuring 100% of the sample is in the detection system and this is the only time the detection medium is opened during the procedure.

The simple one-step procedure allows non-microbiologists to use the system, any positive result confirmed by an external laboratory.

Path-Chek is a more cost effective test as it is a complete kit comprising of swabs premoistened with a wetting agent capable of neutralising any detergents or sanitisers and the chromogenic detection broth.

The media system requires an additional purchase of a collection swab and resuscitation broth which adds to the cost.

Site description	Identification of confirmed isolate
Drain, Line 9	L. monocytogenes
Crack in floor, Line 7	L. monocytogenes
Conveyor, Line 4	Listeria spp.
Crate conveyor, Line 9	L. monocytogenes
Conveyor, Line 3	Listeria spp

Table 2. Identification of confirmed positive isolates from each site.

Conclusion

This study indicates Path-Chek Listeria was the most sensitive, easy-to-use rapid (24-48 hours to result) in-house method for the detection of listeria, which will result in a significant time and cost saving compared to an external laboratory and assist in preventing serious health problems for consumers and expensive recalls for manufacturers. ■

FaxNOW +44 1276 600151
✉ info@microgenbioproducts.com

We wish to acknowledge and appreciate the work of David Wong, Scientific Services Manager at Dairy Farmers, Australia, in the production of this data.

Table 1. Summary of listeria detection using three testing methods used on five sites on five separate occasions.

Site Description	Test Date 4/03/2009 Result	9/03/2009 Result	11/03/2009 Result	16/03/2009 Result	18/03/2009 Result
Path-Chek					
Drain, Line 9	Positive	Positive	Positive	Positive	Positive
Crack in floor, Line 7	Positive	Positive	Positive	Positive	Positive
Conveyor, Line 4	Positive	Positive	Positive	Negative	False Positive
Crate conveyor, Line 9	Positive	Negative	Negative	Negative	Negative
Conveyor, Line 3	Negative	Negative	False Positive	False Positive	Positive
Media based system					
Drain, Line 9	False Negative	False Negative	False Negative	False Negative	False Negative
Crack in floor, Line 7	Positive	Positive	Positive	Positive	Positive
Conveyor, Line 4	Positive	False Negative	False Negative	Negative	Negative
Crate conveyor, Line 9	Positive	Negative	Negative	Negative	Negative
Conveyor, Line 3	Negative	Negative	Negative	Negative	False Negative
Automated immuno-analyser system (NATA Approved Method)					
Drain, Line 9	Positive	Positive	Positive	Positive	Positive
Crack in floor, Line 7	Positive	Positive	Positive	Positive	Positive
Conveyor, Line 4	Positive	False Negative	False Negative	Negative	Negative
Crate conveyor, Line 9	Positive	Negative	Negative	Negative	Negative
Conveyor, Line 3	Negative	Negative	Negative	Negative	Negative