MICROLAB

Microgen Bioproducts Newsletter Nº 30 2010

Dairy Farmers Confirm the Superior Performance of Path-Chek Listeria

To ensure products are free from microbial contamination food manufacturers are increasingly adopting environmental sampling program as part of their HACCP (Hazard Analysis Critical Control Point) plans. An environmental monitoring programme focused on risk assessment and 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 Listerosis 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 (1). 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.

Environmental Monitoring

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. However, these methods do not and cannot 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 specific 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 sanitizing procedures (2).

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 product residue. ATP analysis is not a microbiological method as it is based on the enzymatic conversion of Luciferin to Oxyluciferin. 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 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 5 days to achieve a final result. The time to result and the 3 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.

The Solution?

Microgen Bioproducts has overcome problems associated with these traditional methods of environmental monitoring by the introduction of Path-Chek Hygiene Pathogen system for the detection of important food-borne 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 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 (Figure 1). 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 (3).

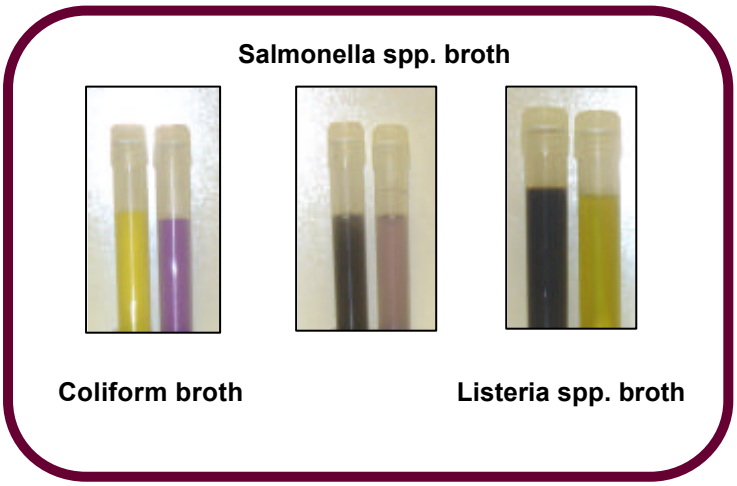

Figure 1. Path-Chek Hygiene Pathogen detection broth. Positive reactions are on the left and negative are on the right.

Evaluation

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. This validation includes parallel testing using bioMerieux VIDAS® from a NATA accredited laboratory (NATA is Australia's internationally recognised laboratory accreditation authority) as well as the 3M Listeria Petrifilm™.

Method

Environmental swabs were collected within one of the Dairy Farmers manufacturing sites. In each area, 3 separate swabs were taken within a 5 minute period to minimise variability and each site was sampled 5 times over a 5 week period. One swab from each site was tested for the presence of *Listeria* spp. using each of the systems under evaluation.

3M Petrifilm™

3M Petrifilm™ *Listeria* 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 (Figure 2).

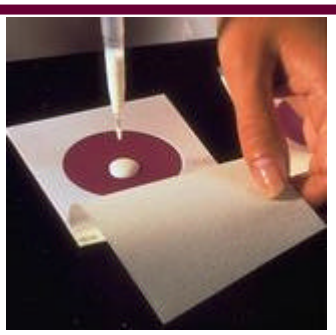

Figure 2. 3M Petrifilm™ being inoculated.

bioMerieux's VIDAS®

bioMerieux's VIDAS® system is an automated qualitative instrument which utilises ELFA (Enzyme Linked Fluorescent ASSAY) technique. Environmental Listeria samples for analysis via a VIDAS system requires 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, 0.5 ml of the fraser broth inoculates the VIDAS® strip, which is then analysed automatically by the instrument and a test value generated for each sample (Figure 3).

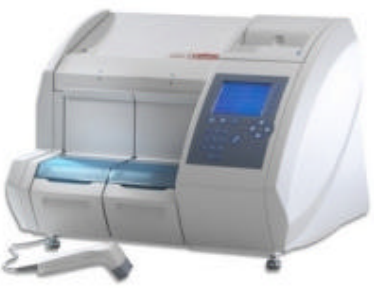

Figure 3. bioMerieux's VIDAS®

Path-Chek

Site Description	Test Date				
	4/03/2009	9/03/2009	11/03/2009	16/03/2009	18/03/2009
	Result	Result	Result	Result	Result
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

3M Petrifilm

Site Description	Test Date				
	4/03/2009	9/03/2009	11/03/2009	16/03/2009	16/03/2009
	Result	Result	Result	Result	Result
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

Siliker (NATA Approved Method)

Site Description	Test Date				
	4/03/2009	9/03/2009	11/03/2009	16/03/2009	18/03/2009
	Result	Result	Result	Result	Result
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

Table. 1 Summary of Listeria detection using 3 testing methods used on 5 sites on 5 separate occasions

Site Description	Identification of Confirmed Isolate
Drain, Line 9	*L. monocytogenes*
Crack in floor, Line 7	*L. monocytogenes*
Conveyor, Line 4	*Listeria* species
Crate Conveyor, Line 9	*L. monocytogenes*
Conveyor, Line 3	*Listeria* species

Table 2. Identification of confirmed positive isolates from each site

Discussion

In this study Petrifilm™ demonstrated a lower sensitivity, with detection of only 47% of the confirmed positive samples using other methods, in comparison to Path-Chek Listeria (100%) and VIDAS® (87%). Path-Chek did have 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 the fact that 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 practice still existed. 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 3M Petrifilm ™ is possibly due to fact that this method does not involve an enrichment step that would increase the overall sensitivity, particularly when a low number of cells are involved or cells have been damaged or stressed by temperature or detergents and sanitizers.

Path-Chek and Petrifilm™ are both low cost in-house methods used for environmental monitoring although Petrifilm™ requires the expertise of a microbiologist as there are two aseptic steps (resuscitation and inoculation). Path-Chek 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 and any result can be confirmed by an external laboratory.

Path-Chek is a more cost-effective test to Petrifilm™ as it is a complete kit comprising of sanitising neutraliser, pre-moistened swab which has the benefit of neutralising any detergents or sanitizers and chromogenic detection broth. Petrifilm™ requires an additional purchase of a collection swab and resuscitation broth which adds to the cost.

This study indicates Path-Chek Listeria as the most sensitive, easy-to-use rapid (24-48hrs to result) in-house method for the detection of Listeria. This would result in a significant time and cost saving compared to an external laboratory, helping prevent a serious health problem and an expensive product recall.

References:

1. Canadian Food Inspection Agency (www.inspection.gc.ca

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