5 TARGETS TO TEST FOR IN YOUR PRODUCTION ENVIRONMENT

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1 Listeria

What is Listeria?

Listeria is a genus of bacteria that are Gram-positive facultative anaerobes found in soil and water. There are up to 17 listeria species with one in particular, Listeria monocytogenes, being a human pathogen. Eating food contaminated with L. monocytogenes causes the disease listeriosis, a serious infection in immunocompromised individuals, newborns, elderly and pregnant women. The illness results in a high rate of hospitalisations and a 15-20% mortality rate. High-risk foods such as ready-to-eat meats, dairy, seafood, fruits and vegetables are subject to post-processing environmental contamination with the pathogen. US regulatory agencies (USDA and FDA) maintain a zero-tolerance policy for L. monocytogenes.

Listeria is killed by normal pasteurisation temperatures but is more heat tolerant than Gram-negative pathogens (such as salmonella and shiga toxin-producing E. coli). Listeria can survive freezing temperatures and is able to grow at refrigerated temperatures. Ready-to-eat foods are of major concern for contamination because they can support the growth of the organism during storage and can be consumed without being cooked.

Where does listeria come from?

Listeria is a very stable organism and can persist in the food processing environment for years. Without proper sanitation and employee hygiene, the pathogen can cross-contaminate both the processing equipment and, eventually, the final product. It thrives in wet, high-salt environments and can form biofilms if not eradicated from the processing environment. Antimicrobial agents (such as pH, moisture level, etc) and antimicrobial processes (such as fermentation, drying, freezing) can suppress the growth of L. monocytogenes in foodstuffs, but the goal is to prevent the inadvertent cross-contamination of the final product from the processing environment.

What kinds of process controls work to prevent listeria?

Traffic patterns, good manufacturing practices (GMPs), sanitary design and sanitation practices are key components to a facility's HACCP and preventive control measures for listeria and L. monocytogenes. Environmental testing is performed to verify the effectiveness of those control programs. If any listeria species are present then there could be a potential growth niche for the pathogenic L. monocytogenes. Food contact surfaces and non-food contact surfaces are tested for listeria species according to a robust sampling program. If food contact surfaces test positive for listeria then finished product testing is required.

What methods do processors use to test?

There are many ways a processor can determine the effectiveness of sanitation and control programs for listeria (including ATP, total plate count, and listeria testing). Rapid methods that test for listeria species (and L. monocytogenes) have proven themselves reliable and are increasing in popularity. Immunoassay-based methods employ antibodies that detect specific proteins, while PCR methods detect DNA. They provide processors with a quick screening tool following the enrichment of environmental samples and food products. A majority of samples will be negative, which allows processors to continue routine operations and release their product faster. Positive test results may indicate potential hot spots or growth niches of listeria in the plant, indicating that corrective actions must be taken to eliminate the source of the contamination before the finished product is compromised. Processors looking to mitigate the risk of product recalls and human illness associated with L. monocytogenes should carefully map their facilities and track the history of listeria on their production line.

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2 Faecal Indicators

What are they?

Testing for indicator organisms is an indispensable component for any microbiological testing program. Though they themselves are not always pathogenic, they may serve to indicate the presence of pathogenic micro-organisms that have made their way into food via improper sanitation or a process failure. Thus, they indirectly provide valuable information about the safety and quality of foods. Faecal contamination of the process environment can include pathogenic organisms such as Salmonella or pathogenic E. coli., among others. There are three micro-organism groups that are commonly identified as faecal indicators:

- Coliforms: Genera that fit this classification are Escherichia, Enterobacter, Klebsiella, and Citrobacter. Coliforms are known to be naturally present in the environment and are not exclusively indicators of faecal pollution.
- Enterobacteriaceae: This family includes 20 genera such as E. coli and all other members of the coliform group, as well as foodborne pathogens like Salmonella, Shigella, Yersinia and other related genera. They are useful in monitoring sanitation in food manufacturing plants, although they are more widely used as indicators in Europe than in the United States.
- E. coli: These are present at high concentrations in all mammalian faeces. They do not multiply in water, but can survive and grow in certain foods. This means they do not necessarily indicate recent faecal contamination.

Although these three groups cannot definitively identify the presence of faecal contamination, testing for them is still a useful monitoring tool.

Where do faecal indicator organisms come from?

While there are multiple sources of potential faecal product contamination, it most often occurs during the slaughtering process. Inadequate personal hygiene on the part of staff can also introduce bacteria into the production environment and product. For fresh produce, the most common cause of faecal contamination is inadequate handling before and during harvesting. If fields are fertilised with cattle manure, the risk of introducing pathogenic E. coli is high, while Salmonella is more an issue in pig and poultry manure. Process water can also harbour faecal organisms.

What type of process controls are utilised?

To minimise the risk of product contamination, a well-established cleaning and sanitising regime must be in effect during production. Proper slaughtering practices can help to avoid contamination from the carcase itself, as can good hygiene practices during the entire production process in general. Between shifts, food-contact surfaces (FCSs) as well as non-food-contact surfaces (NFCSs) should be cleaned and sanitised. To validate these checks, samples should be taken from FCSs on a daily basis, and from NFCSs on a regular basis before, during and after the production run.

What methods do processors use to test?

Quantitative, agar-based cultural methods are the most common methods used for monitoring the processing environment. Generally, there are two ways to conduct agar media-based methods: direct or indirect. With direct methods, plates or dip-slides are placed on the surface to be sampled. These systems are advantageous in terms of handling compared to indirect methods since there is no additional equipment needed and the sampling procedure is very fast. However, the sampling area in this method is limited. Indirect sampling is carried out with swabs, tissues or sponges that are then diluted in a buffer solution before being pipetted into petri dishes and streaked out. The tested surface area can be much broader; tight spaces and gaps can also be tested, but more handling steps and additional supplies are needed. Liquid media-based methods can expand the sampling area even further, but do not allow for quantifiable testing results.

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3 Salmonella

What is salmonella?

Salmonellae are a diverse group of Gram-negative bacteria in the family enterobacteriaceae. There are two species of salmonella (Salmonella enterica and Salmonella bongori). S. bongori is prevalent in cold-blooded animals like reptiles and rarely associated with human illness. Serotypes of S. enterica subspecies enterica (such as S. enterica subspecies enterica Serovar enteritidis and S. typhimurium (for short)) are found in mammals and fowl around the world and in the environment. There are over 2,500 serotypes of Salmonella enterica. These groups of salmonellae are associated with human illness and constitute a major public health concern. A concerning development in recent years has been the emergence of antimicrobial resistant salmonella serotypes.

Where does salmonella come from?

Salmonellae have strongly adapted to poultry. Although affected flocks typically display no visible symptoms, they can cause foodborne illness in humans. To prevent salmonellosis, people are recommended to observe basic food preparation principles such as thoroughly cooking raw poultry products. However, recent outbreaks of salmonellosis have been associated with low-moisture foods (such as peanut butter, spices, and powdered milk). Typically, low-water activity in food creates an environment that is not suitable for pathogens to grow. How, then, are these processed products contaminated? Salmonella contamination has been linked to inadequate sanitation, poor equipment design, lack of proper maintenance, inadequate good manufacturing processes (GMPs) and the introduction of contaminated ingredients into finished products.

What kinds of process controls work to prevent salmonella?

Traffic patterns, GMPs, sanitary design and sanitation practices are key components of a facility's hazard analysis and critical control points (HACCP) and preventive control measures for salmonella. Environmental testing is performed to verify the effectiveness of those control programs. It is common practice to test the production environment and processing equipment for salmonella species. Food contact surfaces and non-food contact surfaces are tested for salmonella species according to a robust sampling program. It is also important periodically to test incoming ingredients for salmonella. Accepting ingredients on Certificate of Analysis (or COA) is no longer sufficient. The United States Food and Drug Administration (FDA) requires periodic testing of incoming ingredients under the Food Safety Modernization Act (FSMA). Contaminated materials, such as powdered milk and spices, can be used as ingredients in a variety of foodstuffs and can lead to massive recalls of numerous commodities.

What methods do processors use to test?

Rapid methods that test for salmonella species have proven reliable. Environmental monitoring provides processors with a tool to verify the effectiveness of sanitation and control programs. A majority of samples will be negative, which allows processors to continue routine operations and release their product faster. Positive test results may indicate potential hot spots or growth niches of salmonella in the plant, indicating that corrective actions must be taken to eliminate the source of the contamination before the finished product is compromised. Processors looking to mitigate the risk of product recalls and human illness associated with salmonella should implement an effective environmental monitoring program (EMP) and routinely test incoming ingredients and finished products for this serious pathogen.

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4 Quality indicator organisms

What are they?

Unlike bacteria, yeasts and moulds are eukaryotic organisms in the fungus kingdom. Most of them belong to the phyla Ascomycota and Zygomycota. Yeasts and moulds are commonly enumerated in foods as quality indicators. They have no predictive value for the occurrence of toxigenic fungi or other pathogens. As a group, the yeasts and moulds are diverse and can grow on virtually any foodstuff.

Where do they come from?

As their spores are airborne, moulds are present virtually everywhere. Yeasts are most commonly used for the fermentation of sugars to alcohol and in the baking industry; the most industrially important strain is Saccharomyces cerevisiae. Yeasts and moulds can survive in a variety of environmental conditions: pH levels can range from 2-9, temperatures from 5-35˚C and water activity (Aw) can be as low as 0.85 or lower. As quality indicators, they can be used to assess the stability and shelf life of a product. Although they have diverse growth habits, yeasts and moulds grow slowly in laboratory culture in comparison to bacteria. Thus, they are enumerated by a plate count procedure that uses agar supplemented with bacteria-inhibiting agents. Chloramphenicol, rose bengal, and dichloran are common selective agents in these agars. Spread or pour plates, incubated at 25˚C for 3-7 days, are recommended (ISO 7954).

Why should I test for them?

The microbes present in a product have a considerable effect on its quality. The types and number of micro-organisms present influence the sensory properties (such as taste, aroma, texture and colour) and shelf life of the product. Food producers may choose to test for some of these micro-organisms for insight into quality changes in the product; for this reason, certain yeasts and moulds are designated as quality indicator organisms. Such quality indicators are often used to ensure that the product is microbiologically stable and acceptable in terms of the sensory properties listed above. The growth and number of a quality indicator is inversely proportional to the quality of the product in question. While the indicator should be present in all products whose quality is to be assessed, its growth should be unaffected by other microbial populations present. There should furthermore be relatively simple methods available for detection, differentiation and quantitation. A good example is yeast and mould counting.

What methods do processors use to test?

Culture techniques rely on growing a particular microbial population to observable levels; this is a time-consuming process and involves producing ideal growth conditions, such as temperature, oxygen content and pressure in liquid or on solid media containing specified nutrients. The resultant colonies may be counted on solid media following incubation. This technique has its drawbacks, as it assumes that each colony is derived from an individual cell and that the incubation conditions would allow for the recovery of all cells present. Despite these limitations, plate count techniques remain the gold standard in qualitative microbiology. Counts may also be achieved in liquid media using most probable number (MPN) techniques. Unlike these more traditional methods, reporter assays assess the microbial population size through the metabolic activity of the cells. The population does not necessarily have to multiply to visible colonies before the measurement takes place. Such techniques include colorimetry; impedance/conductance; ATP-based tests and turbidometry.

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5 Total count (micro-organisms)

What do we mean by total count?

Total count is a very general term that, in this context, requires a more specific definition. Total count normally refers to 'total aerobic mesophilic count'. Sometimes the term 'total viable count' (TVC) is used, but there is no way to count anaerobic and aerobic organisms on agar plates under the same conditions. Furthermore, the term APC (aerobic plate count) is not specific enough as there are different temperature ranges, meaning that a mesophilic plate count (MPC) does not include psychrophiles and thermophiles. Colony forming units (CFUs) and APC (aerobic plate count) refer only to counting methods using agar plates; these are the most common but not the sole methods of total count analysis.

Where do they come from?

As we are talking here about the global micro-organism population, the contamination sources are many and can include:

- Soil and water: Many bacteria are carried in soil and water, which may contaminate food.
- Animal feed: A source of salmonella contamination in poultry and other farm animals.
- Animal hides: Studies have shown that they may be a primary source for E. coli O157:H7, salmonella, and listeria in cattle.
- Gastrointestinal tract: Members of the Enterobacteriaceae group may be found in the faeces of livestock and poultry.
- Food handlers: The microbiota on the hands and outer garments of handlers generally reflect the environment and hygienic habits of these individuals, and the organisms in question may come from hides, gastrointestinal tracts, soil, water, dust, and other environmental sources.
- Food utensils: Saws, cutting boards, knives, grinders, mixers, etc may become contaminated during slaughter and processing operations and are a primary source of cross-contamination.
- Air and dust: A variety of bacteria may be found in air and dust in food processing operations at any one time. Listeria is an example of a Gram-positive organism that survives in the processing environment.

Why should I test for total count?

The real value of a 'total' micro-organism count is derived from historical data trends. If counts are higher than known or accepted numbers, corrective actions may be taken. This very general parameter does not indicate the presence of any harmful organisms, but may help in measuring potential food spoilage. Keeping the total micro-organism count low would result in a lower risk of pathogen contamination and an extended shelf life.

How can I test for total count?

There are many ways to gain information on the global population of micro-organisms in a product sample. Direct counting techniques do not rely on cell growth; one example of these are microscopic analyses in which magnification is used to make individual cells visible. Other procedures for counting micro-organisms have therefore relied on cultivation or metabolic activities. Direct methods are fast but are often labour-intensive or expensive, while cultivation-based methods are slower but affordable. So-called 'reporter assays' are a diverse group and, at moderate cost, can be positioned between those two methods. Here is a brief summary of methods:

- Direct counting: microscopy, automated cytometry.
- Culturing so that the biomass becomes visible: plate count, MPN.
- Reporter assays by which metabolic components are measured: colorimetry, impedance, ATP, turbidometry.

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