Bigger is not always better!

It has been known for over 60 years that swabbing recovers only a small proportion of the total population of bacteria from surfaces, yet many investigators expect precision and accuracy that neither the sample nor the detection method can produce.

Previous articles discussed some of the limitations of environmental surface testing and how these limitations can be mitigated to provide clear actionable results. Variability in the enumeration of low level contamination can be countered with appropriate analysis and binning of results into pass/caution/fail ranges.

Delays in sample collection, transport, storage, and testing can be avoided with well-designed testing procedures informed by microbiological principles. However, some of the issues of testing environmental surfaces are poorly understood and remain unchallenged, for example sample collection methods.

In this article, we will look at some of the issues that affect the recovery and measurement of bacteria from surfaces, and how different sampling methods compare in performance.

The common sampling methods used are a standard cotton swab, wetted with ringers, resuspended in media. More recently large sponge (or cloth) swabs wetted with ringers and resuspended in media have been widely adopted.

An alternative that avoids the need for resuspension of the sample is the use of self-contained devices that provide both the sample collection device and detection material, whereby 100% of the sample collected is tested. Bigger is not always better.

The two key factors related to the sample collection method are:

- Pick-up efficiency – getting the bacteria off the surface and onto the swab/sponge/cloth (sampling device).
- Release efficiency – resuspending the bacteria from the sampling device into the media used for the enumeration test.

The pick-up efficiency of a method depends on the sample area covered, the pressure applied to the surface, the 'stickiness' of the wetting agent and sampling device, the texture and type of surface being sampled, and the degree of attachment of bacteria to the surface.

Moore and Griffiths (2002) studied coliform enumeration from surfaces using different swabbing and detection methods. The surprising conclusion was that sponge swabs were significantly less efficient than all other methods tested.

The limit of detection for traditional swabs and alternative self-contained swabs on inoculated wet surfaces was 1-3cfu/cm^2, whereas it was 200-900cfu/cm^2 for the sponge method. On dry surfaces where viability is markedly reduced, the limit of detection for traditional swab and sponge swabs was 10^4-10^5cfu/cm^2, whereas that of self-contained swab tests was 10^2-10^4cfu/cm^2.

The release efficiency had the greatest effect on swab sensitivity for environmental surface sampling. This appears to be due to resuspension of bacteria that are trapped in or adhere to the matrix of the sampling device such that not all the bacteria collected are available for enumeration by the test method.

Other researchers commented that sponges are very absorbent and have a large internal surface area that repeated compression of the sponge only exacerbates the problem because the released bacteria are quickly reabsorbed and retained.

This would suggest that a self-contained swabbing system will provide more representative results than swab and sponge based methods requiring sample resuspension.

It also begs the question as to the importance of enumerating bacteria from surfaces. ■

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First published in International Food & Meat Topics volume 28.1

Salmonella – an environmental pathogen

Implementing an environment monitoring program for salmonella is a vital preventative measure to ensure food safety.

This genus of >2500 Gram negative bacteria is generally transmitted to humans via consumption of contaminated food such as meat, poultry, eggs and milk but also from plants and seeds. Person-to-person transmission can also occur via the faecal-oral route. Many types of rodents, birds, amphibians and insects are known to be carriers of salmonella and are indirect hazards. In most instances, environmental cross contamination is the cause of an outbreak. It is believed that salmonellosis is responsible for 1.2 million illnesses in the US each year, including 19,000 hospitalisations and 380 deaths. It is also widely accepted that the incidence of food poisoning is unreported by 60-80%.

Symptoms such as diarrhoea, fever, abdominal pain and vomiting usually occur within 12-72 hours and can be debilitating and prolonged. In healthy people the illness is mild with a mortality rate of <1%, however for the very young and old, and immuno-compromised patients the risk of death is 70 times greater.

Salmonella is a ubiquitous and hardy bacterium that can survive for weeks in a dry environment and months in wet conditions. Recommendations for preventing spread are simple; wash hands, cook food thoroughly and wash/peel fruits and vegetables (particularly if being eaten raw). Cross contamination between cooked and uncooked foods should be avoided and food preparation surfaces must be clean.

Finished product testing alone is insufficient to guarantee the absence of salmonella from foods. All food processors should have a specific environmental sampling plan to monitor for salmonella to verify sanitation is effective and minimise the risk of cross contamination. A simple way to achieve this is through the use of a rapid, convenient and affordable screening test kit, such as Hygiena's InSite Salmonella colorimetric test. The importance of environmental monitoring in controlling salmonella is illustrated by some high profile outbreaks, for example:

- In 2008/9, peanut better in the USA affected 714 people with nine deaths as a direct consequence of poor factory hygiene.

- In 2006, a salmonella outbreak in Cadbury chocolate cost the company £20m. It was later found the company had switched from a 'zero tolerance' approach to assuming there could be 'safe' levels of salmonella in chocolate. It reverted after the incident.

- In 1984, a salmonella outbreak from British Airways meals affected nearly 1,000 passengers and staff, and two people died. The source was most likely an aspic glaze contaminated by a chef who had (unreported) diarrhoea.

- A recall of Farley's infant milk in 1985 cost the company £8M and Farley's went into liquidation.

The benefit of environmental monitoring is to identify a problem early and to fix it.

Environmental sampling of direct food contact surfaces and also indirect equipment and facilities offers more relevant information about the risk to the product and enables earlier corrective action.

With cost-effective, simple-to-use environmental screening tests available, food processors and handlers of all sizes are now well placed to perform their own on-site environmental monitoring programme.

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First published in International Food & Meat Topics volume 28.2

Salmonella – detection methods

Last issue, we discussed the importance of testing for salmonella bacteria in food and manufacturing facilities. Environmental sampling plans are essential for the early detection of salmonella outbreaks, to prevent the risk of cross-contamination, verify sanitation, and comply with regulatory standards. Traditional culture methods for detecting salmonella, involving the culture of a sample in a nutrient medium and confirming their growth, can be laborious and time-consuming, often taking 5-7 days to obtain a result.

Over the years, other more rapid methods have been developed. Immunological methods, such as lateral flow device dipsticks and automated enzyme-linked immunosorbent assays (ELISA), involve targeted detection antibodies that bind to the salmonella bacteria, after which a fluorescent reagent or coloured bead typically signals the formation of the antibody-antigen complex. However, these methods still require time to reach the limit of detection of the end point measurement system.

Other molecular-based methods detect salmonella DNA/RNA. For example, in the case of fluorescence in situ hybridisation (FISH), oligonucleotide probes are directed at salmonella rRNA and, after hybridisation, specifically stained cells are detected using epifluorescence microscopy. Another example is polymerase chain reaction (PCR), which makes several copies of salmonella DNA, if present, to amplify it to detectable levels. PCR methods, using instruments such as the BAX System, offer excellent sensitivity, specificity, and accuracy. These detection technologies have a lower limit of detection that effectively reduces the overall time to result.

However, many of these rapid detection methods still require a protracted process of enrichment, and positive results must be confirmed by standardised culturing and biochemical tests in many instances so results may still take 24-30 hours to obtain. Moreover, unless the food processing facility has a laboratory onsite, samples must be sent to external laboratories, forcing reliance on the turnaround times of the supplier. Simple self-contained screening devices, such as InSite Salmonella, quite literally put the control back into the hands of the food processer. This hand-held, all-in-one testing device provides a colorimetric indication of salmonella within just 48 hours, meaning any corrective actions can be taken much earlier. This approach is not only a cheaper and quicker option compared to traditional culture tests, but the only equipment required is a small incubator, so testing can easily be performed on-site (in a secure location) without having to outsource (other than for confirmatory purposes).

To illustrate how simple colorimetric tests can be, the InSite Salmonella enables food processors to screen their facilities for salmonella in just five steps:

- Swab the surface with the integrated cotton bud and incubate the inactivated device at 37°C for six hours.
- Activate the device by breaking the snap valve and squeezing the liquid selective enrichment media into the tube containing the swab.
- Incubate at 37°C for 18-42 hours.
- Check the colour of the liquid media to determine if the test is positive or negative.
- Used devices can easily be disposed of by autoclave, incineration, soaking in 20% bleach for one hour, or via a biohazard disposal facility.

InSite Salmonella is unique because it contains both the pre-enrichment and selective enrichment steps in a single self-contained device such that there are no transfer steps or cross contamination hazards. In addition, a short period of pre-enrichment (6-8 hours) has been proven to provide a faster time to result that also accommodates versatile working practices. Self-contained screening devices provide food manufacturers with an affordable, convenient, quick and simple way of monitoring their on-site environment, to ultimately ensure our food is safe to eat.

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First published in International Food & Meat Topics volume 28.3

Listeria – a risk to food safety

To preserve public health, it is essential for food manufacturing companies to conduct effective environmental screening programmes to control and mitigate Listeria monocytogenes contamination.

L. monocytogenes is a deadly species of foodborne bacteria that, if ingested, can cause the severe infection listeriosis. This can lead to a range of serious health conditions in humans, such as meningitis, gastroenteritis, and bacteraemia, and has a fatality rate as high as 30% in people who are high-risk or immunocompromised, including pregnant women, elderly people, and young children.
L. monocytogenes is ubiquitous in the environment, being naturally found in soil, water and vegetation, as well as being present in animals, such as cattle and fish. It can grow in diverse environments, including in low-moisture environments and at refrigeration temperatures (0-4°C), and can survive harsh salt and pH levels. It can also attach to any food-processing surface, and form biofilms that enhance its resistance to disinfectants and sterilising agents.

Consequently, there are many opportunities for L. monocytogenes contamination during the food production process. Indeed, common causes of transmission to humans have been via consumption of contaminated ready-to-eat (RTE) food products, including raw vegetables, meat and fish, and dairy products, such as ice cream and soft cheeses. Therefore, RTE food manufacturers must comply with regulatory requirements to prevent contamination and transmission to humans. The consequences of an outbreak can be significant. In 2011, Jensen Farms of Colorado, US, was identified as the source of L. monocytogenes-contaminated cantaloupe melons leading to 147 confirmed cases of listeriosis and 30 deaths. After recalling its products and being temporarily shut down by the US FDA, Jensen Farms was declared bankrupt in 2012.
In Europe, the minimum regulatory requirement is to demonstrate that RTE food contains less than 100 colony-forming unit (cfu) per gram of L. monocytogenes at the end of its shelf life but the bacteria are expected to be absent in 25g at the point of manufacture and before release. In contrast, in the USA, the FDA takes a zero-tolerance approach and requires L. monocytogenes to be completely absent. If even low levels are found, the FDA will enforce the complete recall of products, which can have dire consequences for businesses.

It is therefore essential that food manufacturers have environmental screening programmes in place for listeria species and/or L. monocytogenes to enable prompt detection and mitigation of contamination. This can be easily achieved in-house using the self-contained InSite Listeria species test, which detects the presence of any listeria species within 24-48 hours using a simple swab, incubator, and a colorimetric liquid indicator. Further tests are required to confirm the presence and identity of pathogenic listeria (L. monocytogenes). Or, more specific and faster tests such as PCR can be applied to both environmental and product samples.

The zero-tolerance regulatory approach in the US may deter manufacturers from performing sufficient environmental screening tests for L. monocytogenes itself and to focus only on listeria species, in order to minimise the over-zealous and unnecessary demands for recalls. Other countries adopt a more open 'seek and destroy' approach to the measurement of L. monocytogenes in environmental samples. This encourages industry to be more proactive, leading to better surveillance and risk management – and, consequently, greater food safety assurance.

Combining the generic InSite Listeria species test with a specific fluorogenic verification for L. monocytogenes in the same test device will provide QC/QA technicians with a rapid and simple way of obtaining unambiguous evidence of its presence or absence on site. This will enable food manufacturers to avoid unnecessary remedial actions, saving them time and costs, as well

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First published in International Food & Meat Topics volume 28.4

L. monocytogenes – detection methods

We previously discussed the severe consequences of Listeria monocytogenes contamination on human health, as well as on the viability of companies manufacturing fast-moving consumer goods (FMCG) and ready-to-eat (RTE) food products. As such, it is crucial that food manufacturers conduct reliable and systematic environmental screening programmes, designed to detect contamination, verify the effectiveness of control processes and comply with regulatory requirements.

Conventional culture-based microbiological methods for detecting Listeria species are labour- and time-intensive, involving a series of 24-hour long steps to generate the initial presumptive positive result. This then needs to be confirmed through different biochemical tests. Overall, it may take between five and seven days to obtain a final result. Time is of the essence for manufacture and release of FMCG and perishable RTE foods, such that test methods need to deliver timely results in support of controlled storage, despatch and inventory management.

In response, more rapid detection techniques have emerged, such as enzyme-linked immunosorbent assays (ELISA) in automated and dipstick format. These methods use a highly specific antibody-antigen interaction to identify the surface characteristics of the target organism. With an ELISA, specificity is considerably improved and definitive results are generated within two to three days, however, this still is not quick enough.

One of the key reasons that both traditional microbiological techniques and ELISA are so time-consuming is that they typically involve a lengthy selective enrichment period, during which the target organism grows to the required minimum detection level. Moreover, some closely related bacteria that share the same surface or biochemical characteristics make it challenging for these methods to clearly differentiate between the various species. Molecular-based methods offer a powerful alternative because they detect DNA/RNA segments and require fewer target bacteria. Polymerase chain reaction (PCR) is one such method, replicating a characteristic DNA segment to generate millions of copies in as little as one to two hours. This is achieved using special enzymes and substrates with a thermal cycler that exposes the reactants to cycles of repeated heating and cooling. Molecular-based techniques are much more specific and sensitive, while requiring a lower limit of detection and therefore involving fewer and shorter enrichment steps. Some PCR methods may include a shorter end detection step but require several transfer steps and manipulations, whereas others with fewer steps are more robust and take a fraction longer to complete.

Easy-to-use, self-contained screening devices, such as InSite Listeria, allow food manufacturers to save valuable time by having complete control over environmental Listeria species testing.

This post-cleaning verification system generates a presumptive positive result within just 24-48 hours, providing early indication of contamination and facilitating timely undertaking of corrective actions. Contamination can be easily tracked back to its source by taking and processing additional samples.

Using a simple swab, a chromogenic liquid medium selective for Listeria species, and an incubator, InSite Listeria enables testing to be quickly performed onsite without the need for expensive equipment or highly skilled personnel. Additionally, the system is compatible with all detection methods described above. For example, a presumptive positive result could be confirmed using a PCR instrument, such as the BAX System, which offers outstanding sensitivity, specificity and accuracy, as well as rapid time-to-results.

As an all-in-one device, InSite Listeria helps food manufacturers quickly and easily verify the effectiveness of their cleaning processes, and manage the environmental hazards to mitigate the risk of cross contamination from potential pathogens such as L. monocytogenes.

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First published in International Food & Meat Topics volume 28.5

Faster, more reliable approach to listeria detection

There is no questioning the importance of monitoring for the presence of Listeria species, and in particular Listeria monocytogenes, in fast-moving consumer goods (FMCG) and ready-to-eat (RTE) food products. Foodborne illness caused by these contaminating bacteria is a major source of public health concern globally, creating a need for efficient environmental screening programmes, meticulously planned to mitigate the risk of disease outbreaks.

Many current methods for the detection of pathogens such as listeria involve a number of complex and laborious steps to obtain a definitive result. At first, they generate a presumptive positive result, which then needs to be confirmed through a series of biochemical tests to identify the specific isolate. Using traditional culture-based microbiological techniques, this process may take between five and seven days to complete. Enzyme-linked immunosorbent assays (ELISA) and molecular-based methods such as polymerase chain reaction (PCR) are quicker, with the former requiring two to three days and the latter between 24 and 48 hours.

In addition to being time-consuming, complicated and expensive, most of these detection methods are only capable of delivering a single test result following a complete analytical run, screening for either Listeria species or L. monocytogenes.

Food manufacturers can't afford delays in detecting the deadly listeria bacteria; neither can consumers. Latest technological advancements have led Hygiena to develop all-in-one, ready-to-use screening devices for rapid and reliable post-cleaning verification within just 24 hours. These powerful systems combine bioluminescence technology with end-point fluorescence detection in a single device, offering increased specificity for the simultaneous monitoring of environmental surfaces for both Listeria species and L. monocytogenes.

Presumptive positive results are obtained in eight to 16 hours, providing early indication of contamination. This means that food manufacturers can undertake corrective actions in a timely manner, to control the environmental hazards and reduce the cross-contamination potential. Additionally, the technique is capable of screening out the negative samples in just 24 hours, verifying process control procedures and reducing the amount of subsequent confirmatory tests.

Utilising the highly sensitive bioluminescence technology, Hygiena's MicroSnap Surface Express system can detect even very low levels of listeria. The combination of selective bioluminogenic and fluorogenic media effectively reduces false positives, increasing accuracy, precision and reliability of results.

Providing a 2-in-1 screening tool, the technique incorporates a swab and an end detection system, offering a convenient, easy-to-use and cost-effective alternative to more complex detection methods. Testing can be rapidly performed onsite without the need for costly equipment or highly experienced personnel.

The technique retains cell viability and is compatible with all available confirmatory methods. The generated presumptive positive results can be quickly verified using a PCR instrument, such as the BAX System, which can confirm the presence of Listeria species or L monocytogenes within one to three and a half hours.

Offering improved sensitivity, selectivity and specificity, the new all-inclusive method has been designed to provide greater assurance for microbial risk assessment that facilitates the most rapid response. The technology is being developed for even wider applications, such as the detection of total aerobic microbial population and indicator organisms such as Enterobacteriaceae.

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First published in International Food & Meat Topics volume 28.6