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Detecting Sources of *Listeria monocytogenes* in the Ready-To-Eat Food Processing Environment

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The Problem

Listeria monocytogenes is the bacterial species in the genus *Listeria* that causes human listeriosis. Listeriosis, can exist in two forms, including a self-limiting gastrointestinal illness and invasive listeriosis which can be life threatening. The gastrointestinal form is characterized by flu-like symptoms (e.g. diarrhea, vomiting, and fever) that may occur 9-48 hours after ingestion of contaminated food. However, invasive listeriosis may have an onset time of two to six weeks and adults may experience septicemia, meningitis and endocarditis, whereas unborn fetuses may develop abscesses in their liver, lungs and other organs often resulting in spontaneous abortion and still birth. Surviving children may be seriously ill with meningitis and neurological impairment (CDC, 2001; Slutsker and Schuchat, 1999).

Approximately 2,500 cases of foodborne listeriosis and about 500 fatalities occur annually in the United States at an estimated cost of $2.33 billion, making listeriosis the second most costly foodborne illness after salmonellosis (Buzby and Roberts, 1996). Consequently, foodborne listeriosis has been targeted for reduction by many public heath programs, most notably Healthy People 2010 – a comprehensive nationwide health promotion and disease prevention program developed by the Department of Health and Human Services to reduce bacterial infections and enhance life expectancy and quality (http://www.healthypeople.gov/).

Data and personal experience in hundreds of factories (Kornacki, 1999/2000) have demonstrated that recontamination from the processing environment is the principal source of *Listeria* contamination to processed Ready-To-Eat (RTE) foods (Kornacki and Gurtler, 2007; Tompkin, 2002). Scientists, regulators, and food processors have striven for the past two decades to seek out and control *Listeria monocytogenes* in such environments. Progress was evidenced by a steady decline in listeriosis cases from 1996-2001 (http://www.cdc.gov/foodnet/annual/2003/2003_report.pdf). Despite enormous efforts, costs, and early successes by the industry, some believe it is not possible to eradicate this organism from the food processing environment, given currently available technology (Tompkin, 1999). The challenge of controlling this organism is reflected by a leveling off of the incidence of foodborne listeriosis in 2001 and 2002 (CDC, 2004).

Consequently, the food industry must remain vigilant in its *Listeria* control efforts. Control is unlikely without the ability to first find the organism. Questions that should be asked and answered include, "How does one do this effectively?"; "What principles exist to guide my in-factory environmental investigation?"; "What techniques have been effective?; How should I test?" This article will attempt to answer such questions. The following assumes that the plant's HACCP plan(s) has been appropriately validated and verified and Good Manufacturing Practices (GMPs) are being followed.

Where to look

Factory environments are not sterile. *Listeria monocytogenes is very widespread in the natural environment and is* likely to be reintroduced into food production facilities (Tompkin, 2002). They also have properties that permit them to survive and effectively compete with other microbes in food processing environments. Their ability to grow at refrigeration temperatures gives them a competitive advantage over non-psychrotrophic microbes. They are also resistant to freezing and high salt and can adapt to stresses that exist at times in food production facilities (Lou and Yousef, 1999). Some harsh conditions in the factory environment (e.g. acidity, alkalinity) can result in cross protection of the microbe to other stresses, such as heat (Lou and Yousef, 1997; Taormina and Beuchat, 2002).

The areas of greatest relative risk for contaminating food in the factory environment are where *Listeria* has grown to high numbers. These growth niches must be sought and eliminated when found. Many factors affect microbial growth in niches, including moisture, nutrients, pH, oxidation-reduction potential, temperature, presence or absence of inhibitors, interactions between microorganisms in a population, and time (Faust and Gabis, 1988). Areas where water, food (for microbial growth), and time (e.g. areas not accessible for cleaning) combine at a suitable temperature produce microbial growth niches. Nutrition need not be visible to the naked eye to be adequate for microscopic life to grow. Consequently, the best places to sample for *Listeria* are those high moisture environments where the organism has had opportunity to incubate.

It is important to investigate and control conditions that create niches or transmit *Listeria* from them. Elimination of all known niches is important, however, the greatest immediate risk of product contamination will be from those niches and unsanitary conditions which occur after a validated CCP.

Conditions resulting in growth or transmission of Listeria within the processing environment

It is rare, in our experience, that food processing plants deliberately do a poor job of microbiological control. However, unsanitary operating and maintenance/repair practices, and unsanitary equipment/facility design may transmit *Listeria monocytogenes* and/or create such niches in the factory environment. Examples of these are described in Tables 1, 2, and 3, respectively.

Table 1. Selected Examples of Unsanitary Operating Practices

Type of Practice	Unsanitary Deficiency	Concern
Cleaning—general	Inadequate rinse water temperature to liquefy protein and fatty residues	Remaining protein and fat residues will entrap and protect bacteria including *Listeria* from aqueous sanitizer
	Random use of high pressure hoses	High pressure hoses will discharge *Listeria* and other microbes to unreachable areas
	Cleaning equipment in same area as exposed food on operating equipment	Cross contamination from unclean equipment to operating equipment
	Cleaning steps done out of proper order	Ineffective removal of soil resulting in protection of bacteria from sanitizer
	Inadequate number of hose stations	Cross contamination as shared hose is dragged across floor from room to room
	Wet cleaning in dry processing areas	Supplying moisture for creation of microbial growth niches and biofilms
	Insufficient break-down of equipment for cleaning and sanitation	Entrapment of wet residues and formation of microbial growth niches and biofilms on equipment
Cleaning/ scrubbing pads	Reuse of scrubbing pads without adequate sanitation or replacement between uses	Cross contamination of equipment during cleaning
Sanitation—general	Use of alkaline chlorinated cleaner as a sanitizer	Ineffective sanitation as the majority of the chorine is not in the germicidal form due to alkalinity
	Not using the proper sanitizer concentration	Ineffective destruction of *Listeria*
	Flushing processing lines with only water to clean and sanitize	Ineffective removal of *Listeria* biofilms
Cleaning and sanitation—specific		
Sausage peelers	Inadequate cleaning and sanitation may occur (including infrequent cleaning and sanitation)	One *Listeria* contaminated product may cross contaminate peeler gears resulting in growth and subsequent measurable peeled product contamination
Back plate of high pressure pumps	Not broken down for cleaning and sanitation	Contamination of product especially if pump seals are damaged

(continued)

Table 1. Selected Examples of Unsanitary Operating Practices (continued)

Type of Practice	Unsanitary Deficiency	Concern
Slicers	Failure to effectively breakdown slicers for thorough cleaning and sanitation	Development of growth niches and biofilms in entrapped areas may lead to product contamination
	Infrequent cleaning and sanitation of slicers	Development of growth niches and biofilms in entrapped areas may lead to product contamination
Other operating practices/ equipment		
Improper storage of packaging material	Storage of exposed packaging rolls on end on wet floors	Wicking of *Listeria* contaminated residues from floor into the product contact surface of packaging material
	Storage of packaging material in areas exposed to overspray from wet cleaning	Contamination of packaging material with *Listeria*
Storage of product/ ingredients	Improper temperature or time	Recovery of injured *Listeria* and growth in conducive product
Traffic	Traffic from raw to finished product areas	Cross contamination of the processing environment
Brines	Failure to process product contact brine so it remains *Listeria* free	Product contamination with *Listeria*
Improper use of footbaths	Germicide level not monitored or maintained	*Listeria* survival or growth in foot bath resulting in cross contamination of the factory environment by foot traffic
	Inappropriate usage in dry product/processing areas	Inactivation of sanitizer by organic mater may result in creation of growth niches which are dispersed by foot or other traffic
	Failure to effectively clean, sanitize and thoroughly dry after usage (including underside)	Cross contamination of the factory environment
Inappropriate use of gloves in finished product areas	Use of torn gloves or gloves which have touched the floor or equipment/product from raw areas	Cross contamination of product and product contact areas
Inadequately controlled ovens/cookers	Uncontrolled conveyor belt speed through ovens, oven inlet product temperatures, oven humidity, product depth, etc.	Ineffective microbial inactivation

Table 2. Examples of Unsanitary Maintenance/Repair Practices

Equipment or Structure	Improper Practice	Concern
Air handling units	Failure to keep condensate drain lines unplugged	Build-up of cold wet residues facilitating *Listeria* growth in catch pan, possible saturation of final filters in some cases, aerosolization of *Listeria* into factory environment in some cases depending upon design (Table 3)
	Failure to tap in-factory condensate catch pans to drains	Build-up of cold wet residues facilitating *Listeria* growth and contamination of factory environment from overflow
Wheeled ingredient/ product carts	Wheeled product carts with rough welds	Residue build-up facilitating *Listeria* growth and subsequent product contamination
Wheeled, smoked frankfurter trees	Failure to protect exposed product from splashes and aerosols as the trees are wheeled through standing water	*Listeria* contamination of exposed finished product
Unused structures/ equipment	Stored in finished product areas	Such structures may accumulate dust and moisture resulting in a *Listeria* growth niche and environmental contamination
Drains	Failure to prevent back ups or maintain “p”-traps	Cross contamination of the factory environment
Leaking pipes	Duct tape repairs which entrap wet residues	Temporary entrapment of residues allowing for *Listeria* growth and subsequent dripping into the factory environment
Preventative maintenance—general	Not scheduled	*Listeria* may grow on product residues in torn gaskets, insulation, rusted equipment, etc.
Preventative maintenance—specific		
Bearing seals on various equipment	Failure to put these on preventative maintenance program	Entrapment of wet residue and microbes in grease-cross contamination
Torn hoses	Failure to effectively repair or replace	Space between the inner and outer skin accumulates moisture allowing for *Listeria* growth and cross contamination of the factory during cleaning
Freezers, refrigeration units	Torn wet insulation	Wet insulation creates a growth niche for *Listeria* and cross contamination of the factory environment
Vacuum exhaust valves on custom retort (observed by author)	Failure to replace with stainless steel	Retort vacuum exhaust blows contamination from rusted valve unto exposed product

Table 3. Examples of Sanitary Equipment/Facility Design Concerns

Facility or Equipment	Design issue	Concern
	Open trench drains in areas with exposed finished product	*Listeria* growth, splashing, and aerosols. Potential contamination of RTE side of factory
	Drains from raw side flow towards RTE side of factory or drains in finished product area under positive positive pressure from interconnected drains on raw side of factory	*Listeria* from drains, splashing/aerosolizing into RTE side of factory
Processing areas	Raw and finished product areas not adequately separated	Cross contamination
Smokehouses	Common exist and entrance area	Cross contamination potential
Air handling	Positive pressure in raw areas in relation to finished product areas	Airborne cross contamination of the factory environment
Air handling units	Final filter located ahead of cooling coils	Microbial growth on cooling coils and aersolization into the factory environment
Equipment (including cleaning tools)—general e.g. squeegees, threaded scraper attachments	Not easily dissembled for cleaning and sanitation between uses	Growth niche/biofilm development, cross contamination
Product fillers	Some not readily disassembled for their own cleaning and cleaning/sanitation between uses	Growth niche/biofilm development and product cross contamination
Fibrous or cloth areas on product conveyors	Fibers or cloth absorb moisture from wet cleaning and entrap product residue	Cross contamination of product from conveyor
Hollow rollers	Hollow places in conveyor rollers will collect product residue	Difficult to clean resulting in microbial growth niche development, biofilm formation and conveyor belt cross-contamination
Finished product carts	Sharp not rounded inside edges for ease of cleaning/sanitation	Accumulation of product residue and microbial growth/biofilm development
Refrigerators/freezers	Inadequately controlled cooling	Microbial growth in conducive product from resulting from inadequate refrigeration

(continued)

Table 3. Examples of Sanitary Equipment/Facility Design Concerns (continued)

Facility or Equipment	Design issue	Concern
Freezers	Often observed numerous sites for product accumulation	May be difficult to clean/sanitize, microbial growth/biofilm formation in defrost cycle, ineffectiveness of freezing to kill *Listeria* Sloughing of *Listeria* contaminated residues into product during operation
Hollow support structures	These may entrap moisture and become microbial growth niches	Cross contamination of the factory environment
Equipment support structures with large bases bolted to the floor	These entrap moisture are inaccessible for cleaning/sanitizing and become microbial growth niches	Cross contamination of the factory environment
Slicers	Numerous sandwiched areas in some slicers may render them difficult to adequately break down for cleaning and sanitizing	Entrapped residues may result in *Listeria* growth niches/biofilm formation and product contamination with *Listeria* during operation

Routine monitoring

Zones

A number of food processors have found it helpful to beak down their routine environmental sampling program into four zones (Hall, 2004; ICMSF, 2002, Kornacki and Gurtler, 2007). Zone 1 samples are those taken from direct and indirect (e.g. overhead pipes) product contact areas. Zone 2 samples are surfaces adjacent to Zone 1 and include areas like equipment framework and guards. Zone 3 includes surfaces in RTE product zones such as floors, drains, walls, equipment. Zone 4 areas are more remote from the ready-to-eat product zones such as warehouses, loading docks, employee break rooms, and locker rooms. Rotating sampling sites at an appropriate frequency will result in covering a wider region of the factory environment.

An Approach to In-factory risk assessment

The probability of RTE product contamination is affected by a number of variables including but not limited to a.) proximity of microbial growth niches to the product stream, b.) number of growth niches, c.) spatial relationship of niches to product stream, d.) microbial populations in niches, e.) extent of niche disruption, and f.) exposure of product stream to the environment (Faust and Gabis, 1988). Consequently, our investigational approach has been to break down our factory observations and sampling

into regions of "high", "medium" and "indirect" relative risk of product contamination. Areas of high risk are somewhat analogous to Zone 1. These exist where moist, entrapped (or standing) residues are located in close proximity to the product stream. Such an area might include the back plate of a poorly sealed positive displacement pump used to remove product from a heat exchanger (e.g. pasteurizer), or residues entrapped in poorly designed valves located subsequent to a validated Critical Control Point (CCP) in the process stream.

Indirect risk samples would include those from Zones 2-4 that do not produce an *obvious* direct risk of product contamination. However, the microbial ecology of food processing environments is so dynamic that one does not always readily observe the connection, say between a forklift with *Listeria* contaminated wheels observed in a raw processing area, but later charged in a common area with forklifts dedicated for use in ready-to-eat product production (RTE) areas (thereby cross contaminating the wheels of the RTE forklift(s) from the floor). Cross contaminated rotating RTE area forklift wheels may later splash (or aerosolize) *Listeria* onto exposed product.

We defined "medium risk" areas as those places similar to high risk areas, but before some process or procedure with likely potential to reduce the microbial load by an undetermined amount. These usually require a challenge study or process validation to determine the lethality and risk. Medium risks may also be areas or practices which *might* result in contamination of the product infrequently.

One example of a medium risk site may be exposed, and cooling, 135^{o}F molten cheese product in an area with potential for contamination (e.g. ceiling water marks over the product or near employee cross traffic through wet floor areas). This is clearly not a desirable situation and in the limited context of a risk assessment walk-through, would be ranked as a high risk, if the product were not heated. Immediate corrective action to eliminate the contamination potential would still be recommended. In this example, it is not clear if the process temperature and time is adequate to sufficiently destroy a population of *Listeria monocytogenes*. The product matrix plays a significant part in bacterial heat resistance (Stumbo, 1965). Therefore, knowledge of *Listeria* monocytogenes heat resistance over a range of temperatures *in this product matrix* is also needed. If this is not known, a laboratory based thermal challenge study with a multi-strain cocktail of *Listeria monocytogenes* or perhaps a pilot plant based study with appropriate surrogate microorganisms could be done (Kornacki, 2002; Eblen, 2005). These types of studies may result in discovery of a previously unknown CCP. Criteria for selection of surrogates have been described by the FDA (Anonymous, 2000). USDA has also emphasized the importance of process validation studies (Engeljohn, 2004).

Environmental Sampling Considerations

Pre-operational samples (product contact surfaces)
Pre-operational samples are useful to verify effective sanitation of food contact surfaces. USDA indicated that suitable indicator tests such as "*Listeria-like*" organisms or "*Listeria species*" can be used (USDA, 2003). This approach encourages factories to

aggressively sample Zone 1 product contact surfaces, document effective sanitation, and take corrective action in the event of positive data. It is prudent to test finished product for the presence of *Listeria monocytogenes* using an appropriate statistically based sampling plan, if such indicators are found. In this event, an in-factory risk assessment and hold and test product sampling would also be warranted until microbial control is regained.

Testing samples of product contact surfaces for their aerobic plate count (APC) and coliform count is also recommended as indicators of sanitation efficacy, but cannot be relied upon as an indictors of a pathogen (Kornacki and Johnson, 2001).

Operational or Post-Operational Samples

Food processing plant environments are highly dynamic with variations of activity, including traffic patterns, cleaning steps, processing steps and times, airflow, temperature, moisture/relative humidity, nutrition for microbial growth, competitive microorganisms, etc. These factors influence microbial growth and transmission through the plant environment. Consequently, food processors are encouraged to aggressively study the microbial ecology in their factory with particular reference to *Listeria* species or *Listeria*-like organisms on product contact surfaces and *Listeria monocytogenes* in the general (non-product contact) environment. The bulk of sampling should be taken >3 hours into production.

Understanding where and when *Listeria* species, *L. monocytogenes*, and microbial growth niches (e.g. determined by APC) occur in a processing facility can provide valuable information regarding where and when appropriate interventions should occur. Recovery of *Listeria* species, even in the absence of *L. monocytogenes*, is indicative of the potential for *L. monocytogenes* to occur there. A statistically significant correlation between *Listeria* species and *Listeria monocytogenes* was found in the environment of four smoked fish and shell fish processing plants (Thimothe et al, 2004). Regular frequencies and locations for sampling should be established but latitude given for taking investigational samples. Diligence is a key element in seeking and controlling this microbe.

Techniques for Sampling the Environment

A number of techniques exist for sampling the factory environment (Evancho, et al, 2001). More commonly used approaches include the use of pre-sterilized, inhibitor-free sponges, traditional swabs, and contact plates. A comparison of the advantages and disadvantages of these approaches is represented in Table 4. In more recent times the use of 1-Ply composite tissue have shown to provide another effective alternative that may provide a lower cost alternative to the above (Vorst, et al, 2004).

Sponges and swabs must be pre-hydrated with sterile neutralizing buffer before sampling. Hydration of the swab or sponge facilitates greater microbial recovery from a surface and neutralizing buffer is used to prevent residual sanitizer in the sample from destroying the target organism prior to testing.

Table 4. Selected Environmental Sampling Approaches Comparisons

Sampling Technique	Abilities to Effectively Perform Analysis		
	Sample Irregular Surfaces	Qualitative Assays	Sample Heavily Soiled Surfaces
Traditional swabs	Yes	Yes	No
Sponges	Yes	Yes	Yes
Contact plates	Yes	No[a]	No
Tongue blades	Yes	Yes	Yes[b]

[a] May be possible with non-traditional approaches

[b] Biofilm removal more likely

Finished Product/In-Line Sampling

Finished Product Testing

Finished product testing *cannot* be relied upon as the sole determinant of a *Listeria*-free product. No amount of finished product sampling and testing short of assaying the entire product with a perfect method can guarantee that the product is *Listeria*-free. Finding a problem through finished product testing is likely in situations where the incidence of product contamination is high (Table 5). However, this is rarely the case in the United States. For example, *L. monocytogenes* was recovered from 1.6% of 32,800 packages of frankfurters using a method six times more sensitive than the standard USDA/FSIS product composite enrichment method (Wallace, et al, 2003a). Tompkin (2002) stated that "…it should be possible in most food processes that include a validated listericidal step (e.g. cooking) to keep the prevalence of product contamination <0.5%." It is impractical to test enough samples to gain high confidence of detecting contaminated lots with such low contamination incidences. Consider a product contaminated at the 1% level. In theory, 299 randomly selected samples ***per lot*** are required to gain a 95% chance that at least one sample would test positive (Table 5; Midura and Bryant, 2001). If the true incidence is 0.1% it would take 2996 samples ***per lot*** and so forth. Therefore any finished product testing should be viewed as part of a comprehensive *Listeria*-control program including Good Manufacturing Practices, HACCP and its other prerequisite programs. Knowing where to look and taking appropriate environmental samples and appropriate corrective action is far more effective than extensive product testing.

In-line sampling

Sometimes it is impractical to sample product contact surfaces of some processing equipment. We have found rigorous application of statistical sampling techniques at selected locations before and after an inaccessible area has been effective in isolating areas of product contamination.

Table 5. Relationship Between Incidence of Microbial Contamination and Potential For Recovery

Test Number Needed to Detect One or More Positives per Lot			
Percent Positives	**Number of Analytical Units to Be Tested (n)**		
% Positive	**90% Confidence**	**95% Confidence**	**99% Confidence**
100	3	4	4
10	23	30	46
1	230	299	461
0.1	2,303	2,996	4,605
0.01	23,026	29,963	46,052

Note: Adapted form Midura and Bryant. 2001.

Sample testing and compositing

Test methods

Numerous conventional and rapid assays exist for the recovery of *Listeria.* It is best to use those approved by the appropriate regulatory branch in your industry (FDA or USDA Hitchens, 2003; USDA, 2005, respectively) or by an Official Method of Analysis published by the AOAC (http://www.aoac.org/ILM/july_aug_05/oma.htm.). Companies should ensure that methods have been scientifically validated for their particular sample matrix, especially in instances when they need to use a non-approved method.

Compositing product samples

The ability to combine multiple randomly collected samples into one will clearly save testing costs. However, compositing schemes should also be validated. Inappropriate sampling schemes can lead to misleading test results, as described above. The same is true for inappropriate compositing schemes. Some RTE meat sample compositing schemes yielded inconsistent results depending upon the type of meat product sampled and the *Listeria* assay performed (Curiale, 2000).

Other approaches to sampling may also afford enhanced recovery of *Listeria* or reduced labor intensiveness, such as product or package rinses (Wallace, et al, 2003b; Luchansky, et al, 2002).

Molecular subtyping

Once the samples are collected, tested, and isolates recovered a variety of molecular subtyping techniques may be applied such as PFGE, RAPD, RepPCR, and 16s rDNA sequencing. Manufacturers tend to use specialized laboratories for this work, but some have developed in-house techniques for this purpose. In-factory *Listeria* testing is not recommended. These approaches have been useful in revealing specific patterns of *Listeria* transmission that would otherwise not have been understood (Pruett, 2005).

Data Management

Some companies have diligently tracked this microbe and amassed a lot of information. Unless one manages this data properly important trends can be missed. For example, assume *Listeria monocytogenes* was recovered from a site during post-operational sampling. Corrective action is taken and it tests negative at the next sampling. The company might assume effective corrective action occurred. Analysis of trend data throughout the year may tell a different story. Perhaps, the site was positive approximately once per month for 12 consecutive months. Clearly a better corrective action would need to be applied. Eifert (2002) has shown how Pivot tables can be used for precisely this type of analysis.

Summary

Control of *Listeria* in the processing plant environment is an unending task requiring careful thought, vigilance in observation and sampling, diligence in tracking, and appropriate corrective action. Fortunately there are many tools and principles available to the industry.

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