



**Control of *Listeria monocytogenes*
In Mushroom Growing and Packing Environments**

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In recent years, awareness of *Listeria monocytogenes* as a potential microbial contaminant in ready-to-eat foods has increased. Several highly publicized outbreaks attributed to *Listeria* contamination have been traced to consumption of soft-ripened cheese, under-pasteurized milk, and pre-cooked meat and poultry products. This pathogen has also been found in fresh produce including broccoli, carrots, celery, coleslaw, cauliflower, cucumber, green onions, radishes, tomatoes, lettuce and pre-packed salads. To date, there have been no reported cases of foodborne illness attributed to mushrooms contaminated with *Listeria*. However, growers and packers would be wise to understand the unique characteristics of this microorganism and take steps to prevent contamination from occurring.

Listeriosis, the name given to a range of symptoms caused by *L. monocytogenes*, is potentially life-threatening to fetuses of pregnant women, newborns, the elderly and people with weakened immune systems caused by chronic illnesses, such as AIDS or cancer. Less severe cases of listeriosis may result in flu-like symptoms that can include fever, muscle aches, nausea or diarrhea. More severe forms of the disease cause blood infection, meningitis and encephalitis. It is estimated that approximately 20 to 30 percent of severe infections result in death. Infected pregnant women may show little or no evidence of the disease. However, severe cases can lead to premature delivery, spontaneous abortion or stillbirth.

The amount of bacterial cells that must be ingested in order to cause listeriosis has not been clearly established, although it is thought to be relatively low among susceptible individuals. For this reason and because of the potentially severe consequences of the disease, U.S. regulatory agencies have adopted a "Zero Tolerance" policy toward *L. monocytogenes* in ready-to-eat foods. Foods contaminated with *Listeria monocytogenes* are thus considered "adulterated" under the law and can be subject to a recall.

Listeria monocytogenes is found almost everywhere in agricultural environments and has been isolated from 37 species of mammals, both domestic and wild, and 17 species of birds. It is estimated that up to 10 percent of humans may be intestinal carriers.

Unlike most human pathogens, *Listeria* grows well under cool, wet conditions often encountered in growing and packing areas. Therefore, produce buyers increasingly require documentation of monitoring of growing and packing facilities for general cleanliness and sanitation as well as for the presence of *Listeria*. This bacterium is likely to be found in soil, equipment or bins, cull piles and in packinghouses and fresh-cut processing areas. It may be continually introduced into growing and packing areas by employees and by incoming substrate, casing ingredients, and equipment. A well-planned and implemented sanitation program is therefore essential to minimize *Listeria* levels.

Mushroom substrate materials, especially manure-based ingredients, may contain abundant levels of pathogenic microorganisms including *Listeria*. Laboratory experiments conducted at Penn State have demonstrated that a standard Phase II pasteurization and conditioning protocol is effective in eliminating bacterial human pathogens, including *Listeria monocytogenes*. However, storage and handling of unpasteurized substrate must be carefully managed to prevent opportunities for cross-contamination.

By controlling the introduction and growth of *Listeria monocytogenes*, it is possible to minimize or prevent contamination of mushrooms. Because low levels of the pathogen are possible in production and packing environments and, if multiplication occurs, the consequences are so severe, growers and packers should implement Mushroom Good Agricultural Practices in their operations.

Guidelines for monitoring and minimizing the presence of *L. monocytogenes* in mushroom growing and packing areas are provided below. While the material presented here specifically addresses control of *Listeria*, many of these concepts can also be applied to controlling other pathogens such as *E. coli* and Salmonella.

GENERAL CONSIDERATIONS

Control of *L. monocytogenes* can be achieved by both preventing the introduction of bacteria into growing and packing areas and by implementing a systematic cleaning and sanitizing program to reduce levels on equipment, walls, floors and drains. Because the incidence of *Listeria* will vary with each individual operation, managers should develop their own set of procedures and monitor effectiveness by testing for indicator bacteria, such as “generic *Listeria*” to establish a baseline of performance in general sanitation procedures. This monitoring program acts to detect the presence of all *Listeria* species, with the assumption that if any *Listeria* species are detected, *L. monocytogenes* may also be present. Frequency of sampling, location of samples, and necessary corrective actions should be tailored to each plant’s operation. Common sites for this type of cross-contamination are listed below:

- Slicers, dicers, shredders and blenders used after cutting and trimming but before packaging.
- Conveyors.
- Holding containers such as bins, tubs or baskets used to hold the finished product before packaging or further processing.
- Hand tools, gloves and aprons that come into contact with finished product.
- Racks for transporting before packaging.
- Collators used to assemble and arrange product before packaging.
- Filling or packaging equipment.

SAMPLING

Weekly environmental sampling is recommended for most wet areas since these are the locations that are most likely to support survival and growth of bacteria. Drains, floors, walls and overhead and support structures are recommended for sampling. Air sampling may be advisable in some operations but should be done by a qualified microbiologist. After a profile of potential bacteria-harboring sites is established, the plant should develop its sampling schedule accordingly. Any increased incidence in the presence of generic *Listeria* should be further investigated. If a sample that is positive for generic *Listeria* is a composite sample obtained from different sites, the individual samples that made up the composite should be retested to determine where the contamination is occurring. Remember that any contamination during processing will continue to spread downstream and use of that site and all sites downstream from it should be suspended. Intensive cleaning and retesting of the site should be completed.

If generic *Listeria* is detected on a food contact surface, the product should be held until it is determined whether or not it is *Listeria monocytogenes*. If further tests indicate that the pathogenic species is present, the product should be disposed of. If it is negative for *Listeria monocytogenes*, the product may be released. In either case, determine which remedial actions are appropriate, such as modifying cleaning and sanitizing procedures, equipment redesign, and retraining.

Product sampling (whole or sliced and packaged mushrooms) to test for *L. monocytogenes* remains a controversial issue in food safety management and is of debatable value. Initial levels of *Listeria* in the product are likely to be very low and not uniformly distributed. Therefore, the effectiveness of monitoring packed mushrooms in order to assure product safety assurance is questionable. Statistically, unless the final product is grossly contaminated, it is highly unlikely that a practical and economical random sample of finished product would result in a positive detection. Moreover, a strategy to re-sample the product to confirm an initial positive finding has even less validity since the chance of a positive detection in two successive samples is even lower. Remember, if you do detect *Listeria monocytogenes* in your product, FDA considers it to be adulterated and subject to recall.

In-house testing for *L. monocytogenes* is only recommended when appropriate facilities are available for the testing. In addition, adequately trained microbiologists should perform the testing procedures. Many of the test procedures function by steps that amplify the levels of *L. monocytogenes*, if it is present, which requires good laboratory practices to prevent accidental transfer to outside the lab area. Poorly trained individuals or inappropriate facilities could lead to further contamination of the processing facility. It is best to rely on a commercial testing facility that can safely perform these tests.

POSTHARVEST OPERATIONS

A safe general rule is to assume that any breakdown or change made to a facility or packing line might introduce or cause contamination to occur. Examples include the following:

- Postharvest wash water comes from a new, possibly contaminated source.
- A packaging line is moved or changed.
- Used equipment is brought in and installed without thorough cleaning and sanitizing.
- Equipment breakdown leading to the ineffectiveness of some of the barriers to bacterial contamination.
- A drain back-up.
- Product gets caught in newly installed or modified equipment, allowing time for microbial growth in the system.
- Construction in the ready-to-eat product area.
- A new employee is not familiar with the safeguards against *L. monocytogenes* contamination.

Periods of heavy production can lead to a special group of problems. Watch for any of the situations below that may lead to growth and spread of *L. monocytogenes*:

- Personnel are moved from the substrate preparation areas or receiving dock to the finished product area, leading to cross-contamination.
- Busy periods of packaging make it difficult to clean and sanitize as often as necessary.
- Inadequately cleaned product or postharvest equipment in the finished product area.
- Frequent product changeovers.

Here are some basic sanitation guidelines that can significantly limit the possibility of *L. monocytogenes* contamination:

- Remove mushroom residues from harvest and packing surfaces, belts and conveyors, bins and totes.
- Make sure that equipment, parts and product bins and totes are not cleaned on the floor, where *L. monocytogenes* contamination is most common.
- Waste or cull bins in final packing areas should be in good repair, cleaned and sanitized.
- Traffic flow between receiving, packing and shipping areas should be controlled. This includes maintenance employees and outside contractors and their tools. Traffic between substrate preparation areas and finished products areas should be prohibited unless footwear is changed or has been cleaned and sanitized.
- Product flow should proceed in a linear fashion to avoid contact between receiving areas and final packed products.
- It may be beneficial to establish positive air pressure in the finished product area (relative to the raw material side) to contain contamination.
- Compartmentalize. Dedicate separate washing areas for equipment used in substrate preparation areas, growing rooms and final product areas. Color code trash barrels according to contaminated or ready-to-eat areas, use separate utensils and so on.
- Wet process areas should be separated from other areas whenever possible. Bacteria require a cool, damp place to grow. Limiting the amount of standing water helps control the growth of *L. monocytogenes* and most other bacteria.
- Drains from the "soiled" side of packing or processing should not be connected to the drains from the "clean" side.
- Eliminate overhead fixtures in the finished product area wherever possible, especially over areas where the ready-to-eat product is exposed.
- Footbaths can be installed but should be maintained properly. The maintenance of clean, dry floors is more effective. The use of chlorine in a footbath is not recommended because it quickly becomes deactivated. An iodophor or quaternary ammonium compound (Quat) is preferred.
- Post harvest water that comes into contact with the product should contain a sanitizer that approved by the government for use on mushrooms and is effective against *L. monocytogenes*.

PACKAGING AND STORAGE

- Pallets are an often overlooked source of contamination. They should be cleaned and dried before entering packaging areas. Workers who handle mushrooms should not be allowed to handle pallets.
- Packing materials should be palletized and covered until used.
- Cooling units should have dehumidifying properties in order to limit moisture in these areas.

EQUIPMENT

Other areas in growing and packing rooms can provide a place for *Listeria* to grow and contaminate the product indirectly:

- Equipment framework (especially rotating blades, belts, etc.)
- Floors
- Drains
- Walls, especially cracks that retain moisture
- Ceilings, catwalks
- Condensate
- Wet insulation
- Trolleys, forklifts, walk-alongs
- Cleaning tools such as sponges and brushes
- Maintenance tools

Equipment is often forgotten in the scheme of minimizing *L. monocytogenes* risk, but it provides numerous hiding places for bacteria. The following considerations decrease the risk of *L. monocytogenes* contamination of equipment contact surfaces and product surfaces:

- All equipment should be designed to be easily cleaned and serviced.
- Previously used equipment must be thoroughly cleaned and sanitized. Disassemble equipment to clean as needed.
- Maintain equipment in order to minimize breakdowns because the repair of equipment provides an opportunity for the introduction of contamination.
- Hollow equipment or catwalk frames should be prohibited.
- Lubricants should contain a listericidal additive, like sodium benzoate, to prevent them from becoming a harboring point for *L. monocytogenes*.
- Conveyors should not contain hollow rollers and should not be located near the floor. In addition, overhead conveyors should be avoided because they are harder to clean and inspect.
- Transporting carts should have cover guards over the wheels to prevent spray from the wheels from reaching the product.
- If compressed air is used to remove debris from equipment, be sure to maintain and replace in-line filters regularly.
- Coolers or other rooms should not be cleaned when ready-to-eat food is exposed.

SANITATION

Surfaces must be thoroughly cleaned before they can be sanitized. Follow a standard cleaning procedure:

- Dry clean.
- Pre-rinse equipment.
- Visually inspect equipment.
- Foam and scrub equipment.
- Rinse equipment.
- Visually inspect equipment.
- Clean floors.
- Sanitize equipment and floors.
- Conduct post-sanitation verification.
- Dry floors.
- Clean and put away supplies.

Some operations use the following sanitizing protocol:

- After cleaning equipment, apply a high level of chemical sanitizer (800 ppm Quat) and let it sit for 20 minutes.
- Rinse, and then apply a normal level of sanitizer (200 ppm).
- At the end of the week, apply a high level of sanitizer and leave it on equipment until just before start-up.
- Rinse high level of sanitizer and apply a normal level. Then rinse off at start-up.
- It may be beneficial to spray 200 ppm Quat aerosol into a room as a final sanitation step, weekly or monthly.

- The most reliable method of sanitizing equipment is with heat. Heat may be applied to surfaces using hot water (180°F) or steam sprays. However, a good option for tools, utensils, and other small items is to use a COP (clean-out of-place) tank system. Removable slicer heads can be sanitized by completely immersing the pre-cleaned head in hot water. A general recommendation is that the circulating water temperature should be high enough (at least 170°F) to raise all surfaces within the slicer to at least 160°F for 30 seconds. Check with the slicer manufacturer to determine if your equipment is compatible with high temperature treatments.
- Because it may be difficult to measure temperatures deep within a complex piece of equipment, you should validate the effectiveness of sanitization treatment by determining aerobic plate count (APC) populations on slicer surfaces before and after treatment.
- Any time moist heat is used, make sure there is adequate ventilation to remove excess humidity since condensate may develop on ceilings and fixtures and drop onto products.
- Chemical sanitizers such as iodophor (200 ppm) and Quat (400–800 ppm) are effective on equipment and other surfaces. Sanitizers containing peracetic acid and peroctanoic acid are also effective against *L. monocytogenes*. The table below offers some suggestions for cleaning and sanitizing frequencies. Always follow the label directions for maximum allowable levels for no-rinse applications on food-contact surfaces. Regular verification testing will tell you if your treatments are effective against *Listeria*.
- Rotating sanitizers in the program may increase effectiveness.
- Sanitizing with high temperatures may increase effectiveness. But some sanitizers, such as chlorine sanitizers, can be highly corrosive to stainless steel at high temperatures. Read the manufacturers' instructions to judge whether this is advisable with any product.

Packing Areas to Clean with Quat or Peracid	
Area	Frequency
Drains	Daily
Floors	Daily
Waste and storage containers	Daily
Walls	Daily or weekly
Condensate drip pans	Weekly or monthly
HVAC	Weekly or monthly
Coolers	Weekly or monthly

GENERAL PACKING HOUSE SANITATION

- Visual inspection and routine microbiological testing (for example, Aerobic Plate Count) are important in the development of an idea of what potential bacterial problems are present in a facility. Commercial bioluminescent monitoring systems are useful in observing overall sanitation. However, none of these techniques are specific for *L. monocytogenes*. A generic *Listeria* monitoring system is also recommended.
- Clean-up crews should receive special training in controlling *L. monocytogenes*, as well as close supervision. The clean-up crew is most effective if employees understand why sanitizing procedures are necessary. Management and employees should share the view that monitoring is needed to identify needs and opportunities to improve cleaning techniques or frequency in specific areas.
- Mid-shift cleanups should be eliminated when possible, as they produce aerosols and add water to the processing environment.
- Divert condensate from drip pans of refrigeration units directly into a drain. Solid sanitizers should be placed in the drip pans.
- Clean floors daily with an effective cleaning solution. Brushes should be color-coded according to what area they belong to.
- Make sure drains are designed to prevent backups. Stop production if a backup does occur. The room must then be cleaned, rinsed and sanitized. Do not clear a drain with a high-pressure hose, since this creates aerosol droplets that can quickly spread throughout the room.
- Eliminate trench drains where possible.

- Use bactericidal drain rings.
- Brushes used for cleaning drains should be dedicated to that purpose and easily identified through color coding.
- Sanitize cleaning tools with 600 to 1000 ppm quaternary ammonium solution.

EMPLOYEE HYGIENE

- Clean gloves, smocks and aprons are essential. Depending on your operation, color-code these items according to which production area the employee is assigned.
- Make sure employees understand that the clean garments and disposable gloves are to protect the product from contamination, not to protect the employees from getting dirty.
- If an employee touches an unclean surface, their hands should be washed and their gloves changed.
- If possible, have one person responsible solely for picking up material from the floor, removing trash and so on.

CONCLUSION

Listeria monocytogenes is a serious microbial hazard that is commonly found in agricultural environments. Growers and packers of fresh mushrooms should, therefore, consider practices that can protect their products from becoming contaminated. Management needs to set clear policies and train employees so that they understand the importance of proper sanitary practices. Many seemingly insignificant practices, such as moving people and equipment from raw materials areas to finished product areas, storing food-contact containers on the floor, not wearing clean gloves, handling unsanitary utensils or equipment and then touching finished products can greatly increase the chances for *Listeria* contamination to occur. Food safety is everyone's responsibility. Make sure you are doing everything you can to ensure the safety of your products.

ACKNOWLEDGEMENT:

The recommendations in this article are adapted in part from the article "Guidelines for Controlling *Listeria monocytogenes* in Small- to Medium-Scale Packing and Fresh-Cut Operations" by Trevor V. Suslow and Linda J. Harris of the University of California – Davis. Adaptations were made, as necessary, to take into account practices unique to the mushroom industry.

ADDITIONAL RESOURCES

Penn State Mushroom Food Safety Web Site

Located at <http://foodsafety.cas.psu.edu/mush/foodsafety.htm>, this Penn State web site has many links to food safety information including guidelines for mushroom growers and packers and food safety presentations and articles on *Listeria monocytogenes*, avian influenza virus, and other important issues.

Training Opportunities

There are many training food safety training opportunities from academic institutions and private companies. Each year the Penn State Department of Food Science presents the "**Penn State Sanitation Short Course – Prerequisites for Food Safety**". This 2-½ day event, teaches the essentials of food safety including practical guidance on cleaning and sanitizing, equipment and facilities design, and pest control. For details about the program, contact Dr. Luke LaBorde, Department of Food Science, Penn State University, 814-863-2298 or via email at lf15@psu.edu or visit the short course information web site at <http://foodsafety.cas.psu.edu/sanitation.html>.

For those who find it difficult to get away from the office, Penn State now offers "**Food Safety and Sanitation for Food Manufacturers**," a web-based introductory course on food safety and sanitary practices for commercial manufacturers of food products. This course teaches the essentials of food microbiology, sanitary design principles for facilities and equipment, worker hygiene practices, correct procedures for cleaning and sanitizing, food security, and more. This is an "independent study" course delivered through the Internet. That means you work independently and at your own pace; at work, the library, your home, anywhere! For more information, visit the course website at <http://www.foodscience.psu.edu/e-learning.html>.