Listeria, the all-around killer

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1. About the Laboratories

Wynland Laboratories in the Western Cape (Wellington) and NviroTek Laboratories in North-West (Hartebeespoort) joined forces to give the most extensive range of services to customers**.** This among others include the extensive range of soil, leaf, water and nutrient uptake tests (patented NU-Test), a very broad range of residual tests, all at Nvirotek, as well as an extensive range of microbiological and chemical testing at Wynland.

Microbiological testing are done on foodstuffs, water, swabs and wine, while chemical testing are done on soil, leaf and water samples. Water testing includes SANS241, Global Gap, sewerage, irrigation, borehole, and bottled waters while food testing are done on different types of foods (e.g. cheeses, sauces, chocolates, spices, beverages, salads and raw materials).

Wynland Laboratories are a SANAS accredited Laboratory while Nvirotek will obtain its accreditation middle of 2018, but is already working stringently according to SANAS and ISO 17025 regulations.

In the Listeriosis crisis that hit SA in 2017/18 Wynland Laboratories identified well in advance the presence of this pathogenic organism, and in consultation with customers, infected products could be withheld from being distributed to outlets. On the other hand, some "privateers" get their products on the market without being tested. Given the spread of the bacteria (soil, water, air etc.) this poses an immense health risk in especially poor areas where livestock are slaughtered without the necessary safety precautions.

2. Listeria

Listeria monocytogenes (L. mono) is a ubiquitous pathogen known to cause food-contamination outbreaks worldwide. *L. mono* is recognized as an important public health problem in the United States (and for that matter in the world). Listeria is a facultative anaerobic (can grow with and

without oxygen), non-spore forming, rod-shaped, Gram-positive, catalase-positive and oxidase-negative bacterium found widespread in the environment and is most frequently isolated from ready-to-eat foods. It is motile at temperatures of 20-25°C and is not killed by freezing. Growth is arrested altogether, but normal growth will be resumed after thawing. As *L. mono* has the ability to grow at refrigerated temperatures as low as 0°C, this greatly increases its ability to evade control in human foodstuffs. It can also grow under fairly low moisture conditions and under salt stress. This organism have the ability to multiply within host cells and spread from cell to cell.

Listeriolysin O (LLO) is a hemolysin produced by the bacterium *Listeria monocytogenes*. The toxin may be considered a virulence factor, since it is crucial for the virulence of L. monocytogenes. Virulence factors are molecules produced by bacteria, viruses, fungi, and protozoa that add to their effectiveness and enable them colonization of a niche in the host. LLO permits *L. monocytogenes* to break out from the phagosomes into the cytosol without harming the plasma membrane of the infected cell, which allows the bacteria to live intracellularly, where they are sheltered from extracellular immune system factors such as antibodies.

2.1. Strains of Listeria

Listeria comprises of six different species. Only one of these species, *L. monocytogenes* causes human illness. The other species are usually harmless to humans. It has evolved the ability to survive in a variety of different environmental conditions, including moist environments like refrigerators, as well as a variety of stress conditions. Within the *L. mono* species, a multitude of different strains (more than 300 strains identified in one study) have been documented.

2.2. Incidence

Listeriosis is the infection when infected by the bacterium *L. monocytogenes*. Listeriosis is a serious but preventable and treatable disease and usually occurs in people with weakened immune systems, the elderly, pregnant women, and new-borns. Most Listeria cases are reported in high-income countries, while cases are much more likely to go unreported in developing countries. Because of its high hospitalization rate in the U.S., it's the third most costly foodborne pathogen, behind *Clostridium botulinum* (botulism) and *Vibrio vulnificus*. Listeria causes significantly fewer deaths worldwide than Salmonella Typhi (216,500 annual deaths) or non-typhoidal Salmonella (155,000), but it does have a far higher mortality rate. According to The United Nations the South Africa's listeriosis outbreak is believed to be the

largest ever worldwide. The country usually records 60-80 cases a year; however 71 cases had been detected in the Western Cape by November 2017 already and has spread to 750 reported cases in the whole of SA by February 2018. The food-borne disease is now a category 1 notifiable medical condition.

In the European Union listeriosis follows an upward trend, beginning in 2008, causing 2 161 confirmed cases and 210 reported deaths in 2014 (16% more than in 2013). The infection can begin with fever and gastrointestinal symptoms, but can spread to the blood stream and/or the nervous system giving symptoms of meningitis. In pregnant women, *L. mono* can also spread to the fetus, causing premature delivery, miscarriages, or infections of the newborn baby. It expresses a beta hemolysin, which causes destruction of red blood cells.

The disease listeriosis is very rare and affect 1-5 in 1,000,000 people per year in developed countries. However, South Africa and Africa per se is reckoned as developing countries where hygiene is not always practised to the fullest. In people contracting the disease it can be very serious – an estimate of 20% of people with this disease will die. In South Africa this percentage is likely to be much higher. Pregnant women are 20 times more likely to get listeriosis (if compared to healthy individuals), and account for about a third of all listeriosis cases. People with AIDS are about 850 times more likely to get listeriosis, if compared with healthy individuals.

The incubation period is the period from consuming contaminated food until the first signs of symptoms. The incubation period is usually a three-week period and symptoms usually appears within 2-30 days, but can be as much as 70 days after exposure, making it difficult to trace the illness back to the source.

2.3. Avoiding Outbreaks

As said above, Listeria is everywhere. Hygiene is of the utmost importance to try and contain this bacteria. Though it is impossible to completely eradicate *L. mono* from a production plant, steps can be taken to prevent and control the bacterium in order to assure consumer safety while protecting your business. A clean, dry environment is of the utmost importance in controlling Listeria. Common processing facility contamination sites include floors, walls, ceilings, food contact surfaces, cleaning aids, drains/wash areas, heating, ventilation, and air conditioning (HVAC) systems. Potential problem areas should be identified at each processing facility. Among the many general guidelines for keeping a food processing facility clean, visual

inspection and routine testing are important. While regular cleaning can help prevent outbreaks, continuous monitoring is needed to assess further control measures. Good practice includes the determination of sampling points and frequency for routine environmental testing, food contact surface testing, and product testing. Personal hygiene training are crucial and best practice will be to allocate at least one staff member to be responsible for overall facility sanitation.

2.4. Methods of Detection

There are different methods to identify *L. mono* in the laboratory. For example chromogenic agars which claim to be specific for specific bacteria. Unfortunately, some opportunistic bacteria may also grow on the selective media for *L. mono*. *Lycinibacillus sphaericus* is one such culprit that has been identified by Wynland laboratories to grow on specific *L. mono* chromogenic media, and it requires a well-trained microbiologist to interpret the correct result. In order to correctly identify *L. mono* or any other pathogenic bacteria, it is important to make use of alternative methods. Albeit expensive, a human life cannot be measured against expenses. A facility can also be closed down if found to distribute contaminated products.

2.5. Infective Dose

USA standards say <100 colony forming units per gram of food (CFU) of Listeria is safe for consumption. Even though this is USA statistics, a better strategie to follow will be: “rather safe than sorry”. There is, however no threshold below which there is a true “zero” risk for human illness. Estimates based on USA data suggests though that less than 0,2% of the 2500 listeriosis cases that occur annually in the US are caused by food contaminated with 100 or less cfu’s per serving. By contrast, more than 80% of these cases are caused by foods contaminated with more than 1,000,000 cfu’s per serving. The question is, do you have a risk profile and how will you know?

2.6. Listeria in Foodstuff

Listeria can be found in unprocessed food such as raw dairy, meat, poultry, fish, as well as processed foods such as deli meats, hot dogs and cheese - like soft ripened cheeses (eg Brie, Camembert, and Feta) and ice-cream. Soft-ripened cheeses belong to the type of food most often contaminated with *Listeria monocytogenes*. It is also sometimes found in raw vegetables.

It can also spread via an infected product or surface, such as hands or kitchen counters during food preparation, therefore hygiene is of the utmost importance! Listeria is mainly transmitted

to humans as a foodborne disease, but the presence of Listeria in water increases the risk that it will transfer to food," said Dr Jo Barnes, emeritus senior lecturer at Stellenbosch University's faculty of medicine and health sciences. "You have to wash most food and cooking utensils when preparing food. Many food items, such as vegetables and fruit are washed before packaging for selling". She explained that all these hygiene actions mean that Listeria can readily transfer from water to food all the way from the point where the food is produced, to where it is marketed and up to when the food arrives on the dinner table.

Even if *L. mono* is absent, or found at very low prevalence and levels, additional contamination can occur after the packaging of the products are opened. Growth at under 4°C are slow, but above 7°C occurs rapidly, increasing the risk that *L. mono* will reach levels more likely to cause human illness. Both safe handling and maintaining proper refrigeration temperatures are critical to minimize the risk of listeriosis.

2.7. How to get rid of Listeria

2.7.1. Cooked Meat

Listeria is killed by cooking. Thorough cooking the product to 74°C will kill the bacteria. Consumers at high risk for contracting listeriosis should reheat deli meats immediately before consumption.

2.7.2. Deli Plants

All classes of livestock and thus raw meat may contain Listeria. Raw meat must therefore always be treated as potential sources of *L. mono*. Listeria is expected to enter any meat plant with raw materials and personnel, and once inside, it can live almost anywhere – on floors, drains, cooling, ventilation, slicing and packaging equipment. The strategy for the food industry remains one of risk mitigation driven by robust surveillance and sanitation programs. Regular cleaning and sanitation is needed to prevent growth and survival of this organism, including disassembly and deep cleaning in harbourage points of equipment, and contamination of the surfaces in contact with ready-to-eat meats.

2.7.3. Surveillance

Listeria is everywhere, so elimination is almost impossible. Control of Listeria in a manufacturing plant is monitored by a program of regular swabbing and sampling from the plant "environment". There are standard remedial procedures for immediate re-testing any sites that

test positive for Listeria species, including supplementary cleaning protocols. Environmental testing is industry's best practice to detect and manage Listeria in food processing plants. It is more informative than finished product testing, as it points to the source as a target for further sanitation.

2.8. Carriers

Companion animals (pets) and humans can be asymptomatic carriers of *L. mono*. Transmission of *L. mono* by carrier to another person is probably rare, due to the small dose of bacteria received. Equally airborne infections is rare and has never been reported.

2.9. Cleaning Products

A variety of antimicrobial sprays are available to help prevent outbreaks. While some of these products are applied to air conditioning and refrigeration systems to prevent the spread of the microorganism through circulating air, food additive sprays can also be effective against Listeria on ready-to-eat foods. A bacteriophage-based decontaminant was recently approved by the Food and Drug Administration (FDA) for use in food processing facilities. A similar product, also approved by the FDA, is being marketed for use in grocery store meat.

Air-cleaning devices installed in HVAC ductwork offer another option. These products are intended to remove microorganisms and other particles from the air. Some of these systems utilize ultraviolet rays to sterilize air, while others employ High Efficiency Particulate Air (HEPA) filtration to remove airborne particles of a certain size as air passes through.

There is evidence that some strains of *L. mono* may show resistance to certain sanitizers, including quaternary ammonia based sanitizers (contrary to reports to use QA's). In addition and more importantly, *L. mono* can survive and multiply in harbourage points in processing plants. These harbourage points may be places that cannot be reached by sanitizers. Identification and elimination of harbourage points, for example with the help of environmental sampling and testing programs, is thus crucial for controlling *L. mono* in food processing plants.

2.10. Liquid Dehumidification

The chances of contamination increases with wetness in plants and products. Because bacteria are unable to multiply without sufficient moisture, a hygienic and arid environment can support prevention efforts.

Dry bulb, wet bulb and dew point temperatures are often used to determine the state of humid moist air. Dry bulb is the temperature of air measured by a thermometer exposed to the air but shielded from radiation and moisture. Wet bulb is the temperature measured by a moistened thermometer bulb exposed to the air flow. Dew point is the temperature at which water vapour starts to condense out of the air; above this temperature, the moisture will stay in the air.

Liquid desiccant dehumidification systems provide consistent dry-bulb and dew-point temperatures for contamination control. With the ability to handle large moisture loads, such systems provide the low air dew-point temperatures required to maintain dry surfaces and facilitate quick recovery from wash-down.

Buffalo Testing Laboratories Inc., determined the effectiveness of a No-Frost conditioner in removing dust and micro-organisms to be 98% of all air-borne contaminants. Of that 98%, the No-Frost liquid itself removed 99,9 – 100%, depending on the concentration. In addition, the State University of New York at Buffalo department of microbiology also tested antibacterial effects of No-Frost liquids and found that Listeria was inhibited by 99,4 – 99,9% within 24 hours or less, depending on the concentration. Another benefit of the No-Frost system is that all interior parts are readily accessible for inspection and cleaning. The system's interior surfaces are designed to eliminate any pockets that might collect dirt, lint or bacteria.

All the time and resources spent on cleaning are wasted if ventilation systems circulate air containing microorganisms. Combining several available options may be the best way to protect a company, its brand reputation, and its customers.

2.11. The 5 Most Common Places Where to Look for Listeria monocytogenes

2.11.1. Floors

- L. mono is present all around us, mainly on dust on the ground. Ensuring boots are clean in the susceptible areas of the factory is a pre-requisite. This is best done by

changing boots in a hygiene sluice or change room. Use clean, “for inside use only” boots, in your high care environment. Using plastic overshoes is less advisable as it still might give rise to outside dirt setting on top of the plastic. Or even worse: the plastic overshoes might rupture during use and hence you expose your high hygiene area to the risk of *L. mono*.

- Next to management of boots, it is equally important to keep your factory floors dry at all times.
    i. Guiding all water from the production lines in closed pipes to the drain. Do not insert the pipe directly into the drain as this might give rise to bacteria growing from your drain into the pipes. Also make sure you properly clean (preferably CIP) the pipes on a regular basis.
    ii. Make sure the floors slope down towards a drain at all times, placing the drain at the lowest point in your floor. This is the only way to prevent pools of standing water, which are a risk for L. mono and many other problems (eg flies).
    iii. In case of a product spill on the floor do not use excessive amounts of water to flush the spill into the drain. It is much better to clean the spill using a plastic brush and tray and only use water (and if necessary cleaning and disinfection agents) to remove the last remains of product from the floor.

2.11.2. Drains

- One of the locations you will most certainly find *L. mono* when it is present in your factory will be your drains. The main reason for this is that your drains act as a magnifying glass for the biodiversity you have in your factory. This is caused by the fact that most of the time all the dirty water including a lot of the cleaning water is extracted via the drains. Add to this the fact that in quite a few factories the drains are not of a proper hygienic design, and you end up with a perfect situation for bacteria to enter and grow. The best place to start is looking for EHEDG certified drains. These can be found in various places, eg. www.hygienefirst.com and www.blucher.com/technical/hygienic-drainage-design. Next to using a hygienically designed drain it is also important to keep it clean and sanitized at all times. For this reason it is equally important to insert a chlorine tablet in your drain after each cleaning cycle. This way you will keep the standing water in the drain (which will keep the smell out of your factory) clean and sanitized. Each time you have a big spill of

water into the drain outside your normal cleaning regime, you may want to check if the chlorine tablet is still present.

- Flies & Cockroaches. Drains that go uncleaned can easily become breeding grounds for fruit flies, drain flies and cockroaches who feed of leftover food-remains in the drains. These insects carry disease and can spread bacteria from one area to another as they move about a processing plant.

2.11.3. Equipment

- Next to floors and drains you also want to ensure that you do not allow *L. mono* to find places to sit and grow in your processing equipment. Typically all the places you can properly clean and disinfect via your Clean-in-place (CIP) system will be safe. The reason for this is that your CIP can only function properly if all your surfaces are smooth and designed for easy cleaning (which means no crevasses and only smooth surfaces). Places on your equipment where *L. mono* might start building up without being noticed easily, are typically those places that need manual cleaning, having crevasses and often have standing water (or show the marks of standing water like calcite deposits). The closer to the open product you have spots with frequent standing water on your equipment, the bigger the potential risk of introducing *L. mono* into your product stream. The golden rule: keep the water away from your product at all times. If you have places of standing water on your equipment within 2 meters of your open product, the equipment need to be changed and/or the source of water in that location removed.
- Next to the design of the equipment you must be prudent with the use of water any way. You NEVER want to use high pressure water in your hand spraying systems. High pressure water will give rise to aerosols being formed (small drops of water floating in the air). These aerosols are the perfect transportation mechanism for bacteria and might be the perfect way for *L. mono* to proliferate in your factory.

2.11.4. Freezer/Cooling System

- Another well-known area where *L. mono* can hide is your freezer or cooling systems. All freezers and cooling systems have inside evaporation plates (which are the means through which the cold is spread into the room). In the prevention of *L. mono* it is paramount to keep the evaporation plates clean and sanitized AT ALL TIMES. As we know, *L. mono* can survive at very low temperatures and reports show that it may

even grow at temperatures as low as -5°C. For this reason you must ensure you regularly clean and sanitize the evaporation plates and fans of all your freezers and cooling systems.

- Companies producing cleaning agents even have developed specialized cleaning liquids and foams for these types of applications. Just in case your supplier does not have these products, have a look at these websites:
    i. www.goodway.com/coil-cleaning-accessories/coil-cleaner-chemicals-detergents/coilshine-bc-mold-and-mildew-inhibitor-hvac#.WKLUaDvhDDc
    ii. www.ecolab.com/program/coil-flo-coil-cleaning-program
    iii. www.nalco.com/services/coil-cleaning-sustainability.htm

2.11.5. Air Handling System

- Equal to your freezer and cooling systems the air handling system in your factory might use evaporation plates to adjust the air temperature. So all the steps you take for the freezer and cooling systems you need to take for your air handling system as well. For air handling systems there is one other dimension you want to take into consideration as well: the likelihood of water build-up (through condensation) inside the air transportation ducts and the air vents entering your factory. It is wise to execute a boroscope inspection just after a manual cleaning of your factory (when there is a lot of excess water in the air) to see if there is water build-up in your air handling system.
- Next to L. mono, air handling systems are also a well-known mechanism through which Salmonella might enter your factory

2.12. The three step solution to control Listeria

The three steps to control listeria in a food processing environment is, identification, elimination and control

i. Identification: The first step is to identify that a problem exists. If environmental testing is conducted, non-food and food contact surfaces should be sampled. Areas such as floor, sink drains as well as conveyers, slicers, utensils, and other food contact surfaces should be individually swabbed.
ii. Elimination: An important step in controlling Listeria is a good cleaning and sanitation program. Cleaning and Sanitation needs to be a 2-step process that includes cleaning FOLLOWED by sanitation or disinfection. Sanitizing alone

will not remove organic soils, the food source for the organism and soils can interfere with sanitizers and prevent them from functioning. Therefore, Listeria will not be eliminated if drains are not cleaned to thoroughly remove organic soils. The best type of cleaner to use is one that clings to any traces of soil and separates them from the sides of the drains. A cleaner that contains alkalinity and chlorine will have the best performance against soil containing fat and protein as is typically found in floor drains. With the use of a properly formulated cleaner, brushes and pads are not required to clean drains. An effective cleanser can work with a no-touch cleaning process that can greatly reduce the risk aerosolizing soils from the drains. A no-touch procedure is also simple enough and removes enough of the unpleasantness associated with traditional drain cleaning that food processor employees are more likely to clean the drains than ignore them. A final characteristic that a good drain cleanser should possess is that it should not be harmful to the soft metals used to make drains, such as brass, and should be able to be used on any drain type.

iii. Control: After the drain has been cleaned and sanitized, the final step in keeping Listeria out of the food processing environment is to follow the above steps regularly. Cleaning and sanitation of food and non-food contact surfaces needs to occur at least every day, and in some cases more frequently. To help train staff and offer reminders throughout the day, wall charts and instructions with easy to read images can help staff understand cleaning and hygiene practices. Finally, managers can design auditing surveys to keep track of all the cleaning practices required to make sure each area of concern is being properly attended to on a regular basis.

3. Summary

In summary, L. Mono is a very dangerous food born pathogen, and its presence in a food processing facility can lead to high costs, illness and even death. Plant managers need to be familiar with how Listeria contaminates food and pass this information along to their entire staff. With proper education and the proper tools, staff should understand how to identify

listeria problem areas, reduce the risk of listeriosis with proper cleaners and cleaning practices, and keep their work environment controlled against future outbreaks.