LIGHT ON CELLS® DESIGNED BY NBIOTECH

Working Principle

NBiotech Lights on Cells (LOC)



The **NBiotech LOC** lantern instrument is a portable, lightweight device that does not use chemical developers or reagents to detect bacteria in hygienic processes.

It consists of a set of special frequency UV-A LED lamps (which do not burn, are not germicidal, and do not require special PPE-Personal Protective Equipment) or special filters for proper visualization of bacteria in hygienic production settings. The lantern features a combination of special types of LEDs, different wavelengths, and specific filters to produce a unique type of light spectrum.

This special light produced by our lantern <u>reacts with the protein produced by bacteria</u> during their metabolic processes when they adhere to surfaces and form a biofilm.

Blue spots indicate non-viable matter, such as fibers, dust, dirt, and other residues, which do not have a microbial load. Some product residues may appear blue and could turn greenish as they degrade or decompose.

Greenish spots indicate potentially viable contamination. The light emitted by the lantern reacts with the proteins developed by bacteria, as confirmed by reports from USP/UFSCAR.

The reaction caused by the lantern's light spectrum on the protein shows the presence of bacteria. However, it does not confirm whether the bacteria are active (alive) or inactive (dead). It also does not allow for quantification (in Colony Forming Units - CFU) or qualification (naming the type of bacteria). It can however detect not only active bacteria, but also bacteria that is dead or also in sporulation which may not always be detected by ATP swab tests.

There will be various shades of green depending on:

- The type of bacteria (each one develops a specific protein)
- The duration of exposure on the surface
- Environmental humidity
- Environmental temperature

Observation:

The application of acid during the sanitization process, such as peracetic acid, may cause bacteria to sporulate, and they may later desporulate. If Swab or ATP sampling is conducted within 45 minutes after nebulization, it may result in a false negative for both ATP Swab or microbiological analysis, but not for NBiotech Lights on Cells Instrument Lanterns.

Exceptions for greenish color:



Some substances, such as dairy derivatives, yeast, glue, and silicone, may appear greenish. (The latter two are noticeable by touch when you run your hand over the surface).

Practical way to differentiate between **blue** and greenish:

- If you wipe the surface with a glove or cloth, **blue residues** will move from their original spot and shift to another position.
- **Greenish residues**, on the other hand, do not move. In some cases, they transfer to the glove or cloth, leaving a smear behind.

Field Test Data:

A test was conducted on a stainless steel container, where biofilm presence was detected.

- 1. The container was illuminated, and biofilm was visualized.
- 2. Cleaning was performed, followed by the application of *Enztrat* from Higex.
- 3. The container was illuminated again, revealing biofilm residues in corners, welds, deeper scratches, and even on the flat surface.

The Enztrat product worked effectively, but it did not completely eliminate the biofilm, achieving an approximate removal of 80%.

For more details: Biofilm Revealer - BioView by Higex

Netflix Documentary on how Biofilms can Impact our Lives

Netflix Documentary: Poisoned, The Dirty Truth About Your Food.

https://www.netflix.com/br/title/81460481?s=i&trkid=0&vlang=pt&clip=81649484

Documented Real Life Cases of Outbreaks

Listeria Outbreaks USA

https://www.cdc.gov/listeria/outbreaks/index.html / and https://archive.cdc.gov/

2023 Ice Cream

Leafy Greens

- 2022 Enoki Mushrooms
 - Deli Meat and Cheese

Brie and Camembert Cheese Ice Cream

Fresh Express Brand Packaged Salads

Fully Cooked Chicken

Queso Fresco

2021 Dole Brand Packaged Salads

Fresh Express Brand Packaged Salads

Fully Cooked Chicken

Queso Fresco

2020 Deli Meats

Enoki Mushrooms

2019 Hard-boiled Eggs

Listeria monocytogenes Infections

Deli-Sliced Meats and Cheeses

2018 Pork Products

Deli Ham

- 2017 Vulto Creamery Soft Raw Milk Cheese
- 2016 Frozen Vegetables

Raw Milk

Packaged Salads

2015 Soft Cheeses

Ice Cream

2014 Commercially Produced, Prepackaged Caramel Apples

Bean Sprouts

Cheese

Dairy Products

- 2013 Cheese
- 2012 Ricotta Salata Cheese
- 2011 Cantaloupes

Salmonella Outbreaks USA

https://www.cdc.gov/salmonella/outbreaks.html#print 2023

2023 Ground Beef – Salmonella Saint Paul Raw Cookie Dough – Salmonella Enteritidis Flour – Salmonella Infantis
2022 Alfalfa Sprouts – Salmonella Typhimurium Fish – Salmonella Litchfield Peanut Butter – Salmonella Senftenberg
2021 Alfalfa Sprouts – Salmonella Typhimurium Seafood – Salmonella Thompson Onions – Salmonella Oranienburg Italian-Style Meats – Salmonella Infections Prepackaged Salads – Salmonella Typhimurium Frozen Cooked Shrimp – Salmonella Weltevreden

Raw Frozen Breaded Stuffed Chicken Products – Salmonella Enteritidis

Cashew Brie – Salmonella Infections

Ground Turkey – Salmonella Hadar

- 2020 Wood Ear Mushrooms Salmonella Stanley
 Peaches Salmonella Enteritidis
 Onions Salmonella Newport
- 2019 Cut Fruit Salmonella Javiana

Ground Beef – Salmonella Dublin

Papayas – Salmonella Uganda

Kawaran Brand Tahini – Salmonella Concord

Frozen Raw Tuna – Salmonella Newport

Pre-Cut Melon – Salmonella Carrau

Butterball Brand Ground Turkey – Salmonella Schwarzengrund

2018 Tahini Produced by Achdut Ltd. – Salmonella Concord

Salmonella Agbeni Infections

Raw Chicken Products – Salmonella Infantis

Ground Beef – Salmonella Newport Gravel

Ridge Farms Shell Eggs – Salmonella Enteritidis

Chicken – Salmonella I 4,[5]],12:i:-

Raw Turkey Products – Salmonella Infections

Hy-vee Spring Pasta Salad – Salmonella Sandiego

Kellogg's Honey Smacks Cereal – Salmonella Mbandaka

Pre-Cut Melon – Salmonella Adelaide

Shell Eggs – Salmonella Braenderup

Dried Coconut – Salmonella Typhimurium

Chicken Salad – Salmonella Typhimurium

Kratom – Salmonella I 4,[5],12:b:-

Raw Sprouts [Español] – Salmonella Montevideo

Frozen Shredded Coconut [Vietnamese [PDF – 3 pages]] [Español] – Salmonella I 4,[5],12:b:- and Salmonella Newport

2017 Maradol Papayas [Español] – Salmonella Urbana

Maradol Papayas [Español] – Salmonella Newport and Salmonella Infantis

Maradol Papayas [Español] – Salmonella Anatum

Maradol Papayas [Español] – Salmonella Thompson, Salmonella Kiambu, Salmonella Agona, and Salmonella Gaminara

2016 Shell Eggs – Salmonella Oranienburg

Alfalfa Sprouts - Salmonella Reading and Salmonella

Pistachios – Salmonella Montevideo

Alfalfa Sprouts – Salmonella Muenchen and Salmonella Kentucky

Organic Shake & Meal Products – Salmonella Virchow

2015 Raw Sprouted Nut Butter Spreads – Salmonella Paratyphi B variant L(+) tartrate(+) Cucumbers – Salmonella Poona

Pork – Salmonella I 4, [5], 12: i:- and Salmonella Infantis

Raw, Frozen, Stuffed Chicken Entrees – Salmonella Enteritidis

Raw, Frozen, Stuffed Chicken Entrees – Salmonella Enteritidis

Frozen Raw Tuna – Salmonella Paratyphi B variant L(+) tartrate(+) (+) and Salmonella Weltevreden

2014 Cucumbers – Salmonella Newport

Bean Sprouts – Salmonella Enteritidis

Nut Butter – Salmonella Braenderup

Clinical and Teaching Microbiology Laboratory Exposure – Salmonella Typhimurium Organic Sprouted Chia Powder – Salmonella Newport, Salmonella Hartford, and Salmonella Oranienburg

Chicken – Salmonella Heidelberg

Raw Cashew Cheese – Salmonella Stanley

2013 Chicken – Salmonella Heidelberg

Tahini Sesame Paste – Salmonella Montevideo and Salmonella Mbandaka Cucumbers – Salmonella Saintpaul

Chicken – Salmonella Heidelberg

Ground Beef – Salmonella Typhimurium

2012 Peanut Butter – Salmonella Bredeney

Mangoes – Salmonella Braenderup

Cantaloupe – Salmonella Typhimurium and Salmonella Newport

Ground Beef – Salmonella Enteritidis

Raw Scraped Ground Tuna Product – Salmonella Bareilly and Salmonella Nchanga Mexican Style Restaurant Chain – Salmonella Enteritidis

2011 Ground Beef – Salmonella Typhimurium

Kosher Broiled Chicken Livers – Salmonella Heidelberg

Turkish Pine Nuts – Salmonella Enteritidis

Ground Turkey– Salmonella Heidelberg

Whole, Fresh Imported Papayas – Salmonella Agona

Alfalfa and Spicy Sprouts - Salmonella Enteritidis

Clinical and Teaching Microbiology Laboratories – Salmonella Typhimurium

Turkey Burgers – Salmonella Hadar

Cantaloupe – Salmonella Panama Alfalfa Sprouts – Salmonella I 4,[5],12:i:- Shell Eggs – Salmonella Enteritidis Cheesy Chicken Rice Frozen Entrée – Salmonella Chester Frozen Mamey Fruit Pulp – Salmonella Typhi (Typhoid Fever)

Mexican Style Restaurant Chain – Salmonella Hartford and Salmonella Baildon Alfalfa Sprouts – Salmonella Newport

Red and Black Pepper/Italian-Style Meats – Salmonella Montevideo

2009 and 2008

Alfalfa Sprouts – Salmonella Saintpaul

Pistachios – Salmonella (multiple types)

Peanut Butter – Salmonella Typhimurium

Raw Produce – Salmonella Saintpaul

Rice and Wheat Cereals – Salmonella Agona

Cantaloupes – Salmonella Litchfield

2007 Pot Pies – Salmonella I 4,[5],12:i:-

Vegetable Flavored Rice and Corn Snack – Salmonella Wandsworth

Peanut Butter – Salmonella Tennessee

2006 Tomatoes – Salmonella

Typhimurium Salmonella Animal Outbreaks USA

https://www.cdc.gov/salmonella/outbreaks.html#print

2023, 2022, 2021

Small Turtles – Salmonella Infections
 Backyard Poultry – Salmonella Infections
 Pet Bearded Dragons – Salmonella Infections
 Small Turtles – Salmonella Stanley
 Backyard Poultry – Salmonella Infections
 Pet Bearded Dragons – Salmonella Uganda
 Backyard Poultry – Salmonella Infections
 Wild Songbirds – Salmonella Typhimurium
 Small Turtles – Salmonella Typhimurium

https://www.cdc.gov/ecoli/

2022 Frozen Falafel – E. coli

O121 Ground Beef – E. coli

O157:H7 Unknown Food Source – E. coli

2021 O157:H7 2021 Packaged Salads – E. coli

O157:H7 Baby Spinach – E. coli

O157:H7 Cake mix – E. coli

O121 Unknown Food Source – E. coli

2020 O157:H7 2020 Unknown Source 3 – E. coli

O157:H7 Leafy Greens – E. coli

O157:H7 Unknown Source 1 – E. coli

O157:H7 Clover Sprouts – E. coli O103

2019 Fresh Express Sunflower Crisp Chopped Salad Kits – E. coli

O157:H7 Romaine Lettuce – E. coli

O157:H7 Northfork Bison – E. coli

O103 and O121 Flour – E. coli

O26 Ground Beef – E. coli

O103

2018 Romaine Lettuce – E. coli

O157:H7 Ground Beef – E. coli

O26 Romaine Lettuce – E. coli

O157:H7

2017 Leafy Greens – E. coli

O157:H7 I.M. Healthy SoyNut Butter – E. coli

O157:H7

2016 Beef Products – E. coli

O157:H7 Flour – E. coli

O121 and O26 Alfalfa Sprouts – E. coli

0157

2015 Rotisserie Chicken Salad – E. coli

O157:H7 Mexican Style Restaurant Chain – E. coli

O26

2014 Raw Clover Sprouts – E. coli

O121 Ground Beef – E. coli O157:H7

- 2013 Ready-to-Eat Salads E.coli
 O157:H7 Frozen Food Products E.coli
 O121
- 2012 Organic Spinach and Spring Mix Blend E. coli

O157:H7 Unknown Source – E. coli

O145 Raw Clover Sprouts – E. coli

O26

2011 Romaine Lettuce – E. coli

O157:H7 Travel to Germany – E. coli

O104 Lebanon Bologna – E. coli

O157:H7 In-shell Hazelnuts – E. coli

O157:H7

2010 Cheese – E. coli

O157:H7 Shredded Romaine Lettuce – E. coli

O145 Beef – E. coli O157:H7

2009 Beef – E. coli

O157:H7 Beef – E. coli

O157:H7 Prepackaged Cookie Dough – E. coli

O157:H7

- **2008** ef E. coli O157:H7
- 2007 Pizza E. coli

O157:H7 Ground Beef Patties – E. coli

O157:H7

2006 Mexican Style Restaurant Chain – E. coli O157:H7

Fresh Spinach – E. coli O157:H7

Brazil and Other Countries

Ministério da saúde volume 51 nº 32

https://www.gov.br/saude/pt-br/centrais-deconteudo/publicacoes/boletins/epidemiologicos/edicoes/2020/boletim-epidemiologicosvs-32.pdf/view

In Brazil, isolated incidents do not need to be reported, only food-borne outbreaks. As a result, there is limited data available for analysis, and access to it is difficult. "Many sporadic cases, unfortunately, go unnoticed without proper identification," warns the researcher.

Source: Research points to the presence of more invasive and resistant Salmonella in Brazil

Https://www.siempre.com.br/nono/pesquisa-apunta-presencia-de-salmonela-Mai's-invasive-e-Mai's-resistente-no-brasil/

Microbiological standards for food are regulated by Instrução Normativa 60 and RDC 331 of Anvisa, both published in December 2019.

"However, there are industries that use legal requirements as a precaution: if they know a microorganism capable of causing disease might be present in their products, but there is no legislation covering that microorganism, they do not report it. And this happens worldwide, not just in Brazil."

The biologist also highlights the low number of food recalls that become public in the country.

"Here, it is rare to hear about a company having contamination issues. These reports are not very common. How many food recalls do we have in the country? Very few, compared to countries like the United States. There, they recall everything that presents a problem, whether microbiological or not, and it is published, with the information made available to consumers. Transparency is very important."

In her view, consumers also do not demand a different stance because they lack the information to do so.

"As long as we don't have informed consumers or consumer organizations that can push for change, we will remain in this situation." Listeria – Is not reported in Brazil thus being under-reported and under-diagnosed.

2023

https://www.folhape.com.br/noticias/listeriose-infeccao-causada-por-bacteria-que-podeestar-nos-alimentos/288000/

Frequently Asked Questions (FAQs)

1) What is the minimum time required to visualize bacteria?

The minimum exposure time for the bacteria to produce the protein is 4 to 5 hours. During this period, contamination may be present on the surface but not yet display the greenish coloration.

2) Can we quantify (CFU) Colony Forming Units and qualify (determine the type of bacteria)?

No, as we are visualizing their produced protein, it is not yet possible to correlate the colors with the quantity or type of bacteria.

3) Can we determine if the bacteria are active (alive) or inactive (dead)?

No, since we are visualizing their produced protein, it is not possible to determine whether the bacteria are active or inactive.

4) Does ambient light interfere with visualization?

It can, especially direct white light, which is stronger than UV-A and reduces the flashlight's intensity. You can improve visualization by partially blocking direct light above the lantern with your hand or an accessory. Another commonly used option, when permitted, is conducting the visualization at night or by partially or fully turning off the lights in the specific area.

5) What is the purple light that appears during illumination?

It is the color of the LED lamp itself when reflected on polished surfaces, such as stainless steel.

6) What is the orange light?

A very specific <u>fiber</u> occasionally appears <u>orange</u>. However, as a rule, it is considered nonviable, like the blue coloration.

7) Which surfaces can be visualized, and which cannot?

• Easily visualized surfaces: stainless steel, carbon steel, aluminum, plastics, wood, etc.

• Difficult or non-visible surfaces: transparent glass and acrylic, electro-polished stainless steel, and surfaces with adhered iron sulfate. For electro polished stainless steel (EP) this can be mitigated by holding the instrument lantern at a 45 degree angle. The UV glasses provided with the lantern also help to reduce the glare and allow one to visualize the contamination.

8) Are there exceptions to the greenish tones?

Yes, dried glue or silicone residues can appear greenish. For example, if a tank tag adhesive is removed, and glue residue remains, it will appear green under illumination. By touching the residue, you can confirm it is glue, making this exception easy to distinguish, similar to silicone.

9) Is maintenance required? What about UV lifespan?

Yes, maintenance will be required eventually, usually after a year and a half to two years of continuous use.

- The lamps have an estimated lifespan of 10,000 hours of use.
- Rechargeable batteries last about 2 to 3 years.
- Other electronic components require occasional maintenance and calibration at a low cost.

• The glass filter in front of the LEDs is not part of regular maintenance but may crack if dropped or hit, requiring replacement.

10) What are the lantern's usage and battery recharge times?

Usage time: Up to 5 hours of continuous use. Recharge time: Up to 2 hours.

11) Is it waterproof?

No, it is enclosed and sealed in order to prevent the entry of vapor and liquids, but it does not have a waterproof enclosure certification.

12) Does it have a classified area certification or explosion-proof certification?

No, it does not have such certification. We are beginning a study to evaluate the technical feasibility, costs, and demand in these areas.

13) What are the lantern's dimensions and weight?

- Lantern: 185 x 138 x 156 mm, 1.37 kg
- Case: 1.435 kg

• Charger: 0.35 kg

15) Is it possible to see bacteria in a liquid medium?

It is not common to observe color changes in liquid samples. Exception: E. Coli, Staphylococcus Aureus, Pseudomonas Aeruginosa, and Candida Albicans may exhibit visible reactions.



16) Is it possible to see the greenish tone when bacteria are cultivated in a petri dish with a culture medium?

No, because in a favorable medium, bacteria do not develop their protective surface with MCBs and proteins. However, if the bacteria are transferred to a sterilized stainless steel plate, they will develop over time and typically display the **greenish** tone.

17) Was the fungus Trichoderma detected with fluorescence in the agricultural biotechnology plant?

Yes, it was illuminated both as a powder and in liquid residue, displaying colors ranging from yellowish to rust colored.

18) What is the detection accuracy of the method?

The accuracy of illumination compared to field test analyses averaged 90%. This percentage could be higher; however, the greenish tone only indicates the presence of proteins in the protective surface, not whether the contamination is active or inactive.

Advantages of NBiotech Light on Cells Technology

- Visual identification of contamination sites: Enables the naked eye to locate areas of contamination. <u>Compliments ATP Testing to improve reliability</u>.
- Precise localization of contamination: Pinpoints the exact spots where contamination is present.
- Support tool for ATP and microbiological analyses: Increases the accuracy of sample collection locations.

- Reduction in ATP luminometer and microbiological analysis consumables: Optimizes the use of resources and reduces costs.
- Instant verification: Allows for checks before, during, and after Cleaning-Out-of-Place (COP) processes and surface, instrument, and equipment sanitization.
- Optimization of cleaning processes: Adjusts cleaning times, resources, and labor based on detected dirt and contamination.
- Improved contamination control and cleaning: Enhances product quality and shelf life.
- Infection prevention in hospital environments: Improves contamination and cleaning control in critical areas like ICUs and surgical centers, reducing infection risks.
- Increased safety for patients and professionals: Promotes a safer environment in industries and hospitals.
- Better cleaning inspection and control: Improves cleaning quality and the removal of contamination sources, potentially leading to:
- Reduced hospitalization times.
- Decreased use of antibiotics.
- Improved environmental conditions, reducing hospital-acquired infections and preserving more lives and health.