

# AquaSpark™ – Rapid Environmental Monitoring of *Listeria monocytogenes*

Giverny M. Ganz, Lukas Reinau, Anne-Flore Imhaus, Mario Hupfeld\*, and Lars Fieseler

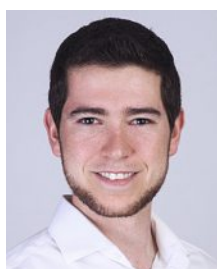
**Abstract:** In order to prevent microbial contamination of food, monitoring of the production environment, together with the rapid detection of foodborne pathogens have proven to be of utmost importance for Food Safety. Environmental monitoring should detect harmful pathogens at the earliest point in time in order for the necessary interventions to be taken. However, current detection methods fall short with regards to speed, ease of use, and cost. This article aims to present the idea behind NEMIS Technologies, a startup company making use of the novel AquaSpark™ technology for the development of a new generation of bacterial detection methods. These methods utilize chemiluminescence in order to detect live target bacteria in a short period of time compared to that of conventional methods. We show that dry-stressed *Listeria monocytogenes* can be detected within 24 hours, using small-molecule chemiluminescent probes, together with a bacteria-specific proprietary enrichment broth containing a cocktail of bacteriophages, which enhance the specificity and sensitivity. This novel platform technology has the potential to extend beyond environmental monitoring towards food analyses as well as veterinary and human health.

**Keywords:** Bacteriophages · Chemiluminescence · Environmental monitoring · Food safety · *Listeria*



**Giverny Ganz** studied microbiology and immunology at ETH, Zürich and completed her Master degree in 2019 focussing on the complex interactions taking place in the host during a *Salmonella Typhimurium* infection. She then worked in the microbiology laboratory for the Canton of Zug, where she assisted in food and water testing for spoilage and hygiene indicators as well as the beginnings of a project involving

the prevalence of antibiotic-resistant bacteria in the environment. She is currently working as a scientific assistant in the Food Microbiology group at ZHAW, Wädenswil as well as for NEMIS Technologies AG on the development of novel microbial detection methods.



**Lukas Reinau** studied Microbiology at the University of Zürich. In his Master thesis, he participated in a project with the long-term goal to develop a new vaccine against tuberculosis. He focused on different proteins of the causative agent *Mycobacterium tuberculosis*, which play a major role in virulence. After completing his Master in 2018, he started his current position as scientific assistant in the Food Microbiology

group at the ZHAW. In his project, he is developing a new detection method for the food pathogen *Listeria monocytogenes* in collaboration with the startup NEMIS Technologies AG.



**Anne-Flore Imhaus** worked in the laboratory of Guillaume Dumenil in Paris, France, where she investigated the molecular mechanisms by which type IV pili exert their functions during *Neisseria meningitidis* infection for her PhD. As a postdoctoral fellow in Sam Miller's group at the University of Washington she studied the role of the antimicrobial peptide sensing by the PhoP-Q two component regulatory system in *Salmonella Typhimurium* infection. She is now working at the Food Microbiology group at ZHAW, Wädenswil to develop a fast and innovative detection kit for Salmonella in collaboration with NEMIS Technologies AG.

function of CSO.



**Mario Hupfeld**, born in Bremen, Germany, studied at the University of Konstanz Life Sciences. He joined the laboratory of Prof. Martin Loessner at ETH Zurich (Food Microbiology) to investigate *Listeria* bacteriophage–host interactions and genetic engineering for his PhD. Early 2018, he co-founded NEMIS Technologies AG where he is responsible for the technical development of the diagnostics kits in the

function of CSO.



**Lars Fieseler** graduated in Biology at the Ludwig-Maximilians-University of Würzburg, Germany, where he also received his PhD. After being awarded with a Feodor-Lynen Research Fellowship from the Alexander von Humboldt Foundation, Germany he joined the group of Prof. Martin J. Loessner at ETH Zurich, Switzerland. He is currently leading the Food Microbiology Research Group at Zurich University of Applied Sciences, Switzerland.

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## 1. Introduction

Foodborne diseases constitute one of the most significant public health concerns throughout the world.<sup>[1]</sup> Food can be contaminated by various microbes, which can further be transmitted to humans, leading to recurrent outbreaks of foodborne illnesses.<sup>[2]</sup> In a report published by the WHO European region, it was estimated that more than 23 million people fell ill from consuming contaminated food in 2010.<sup>[3]</sup> Contributing to these numbers were the common foodborne pathogens *Campylobacter* spp., *Salmonella* spp., pathogenic *Escherichia coli* and *Listeria monocytogenes*, among others.<sup>[4,5]</sup>

*L. monocytogenes* is an opportunistic pathogen that causes Listeriosis, a severe illness and leading cause of death due to foodborne illnesses.<sup>[3]</sup> Outbreaks caused by *L. monocytogenes* as well as recalls of food products can often be traced back to contamination sources in the production environment and the equipment of the food processing facilities.<sup>[6]</sup> Thus, a properly established environmental monitoring programme of food production facilities is crucial as an early warning system for potential microbial hazards. The hazard analysis critical control point (HACCP) concept is an internationally recognized process control system, implemented in commercial food production operations, involving the detailed examination of each step in the preparation of food.<sup>[7]</sup> This concept consists of two pre-requisite programmes, namely, the ‘good manufacturing practice’ (GMP) as well as the ‘good hygiene practice’ (GHP). The GHP system is used in order to assess any potential microbiological hazards, by the detection of dangerous pathogens, as well as indicate whether food and non-food contact surfaces have been cleaned and disinfected correctly, in order to prevent the contamination of food.<sup>[8]</sup>

The traditional methods used to detect *L. monocytogenes* in food or on environmental surfaces, are tedious and can take up to one week until a result is obtained.<sup>[9]</sup> This period of time is unfavorable when it comes to tracing back the product to its production line and recalling the product that may have been contaminated. Particularly products with a short shelf life can land on retail shelves before a test result can be obtained. The current, gold standard, methods for the detection of *L. monocytogenes* in food make use of different enrichment cultures and plating on selective agar over a period of one week in order to obtain a definitive result.<sup>[10,11]</sup> The limitations of this method include the time-consuming aspect and the need for fully equipped laboratories to carry out the procedure.

Due to the high demand for rapid alternative methods, many researchers are developing novel techniques to overcome the limitations imposed by traditional detection methods. There are three categories of rapid alternative methods, namely, nucleic acid-, biosensor- and immunological-based.<sup>[2]</sup> Although the detection time for these methods is considerably reduced to range from a few minutes to hours, a bacterial pre-enrichment of 16–48 h is still required.<sup>[12–14]</sup> Another drawback is that nucleic acid-based detection methods target genotypic features, meaning that a positive result will not indicate the viability of the target bacteria.

Therefore, a pre-enrichment or additional confirmatory tests are required, which will add to the time needed to obtain a definitive result.<sup>[12–14]</sup> Thus, there is a need for rapid, on-site detection of viable bacteria in a factory setting. The ability to carry out testing on-site would reduce costs, save time and eliminate the need for external laboratories.

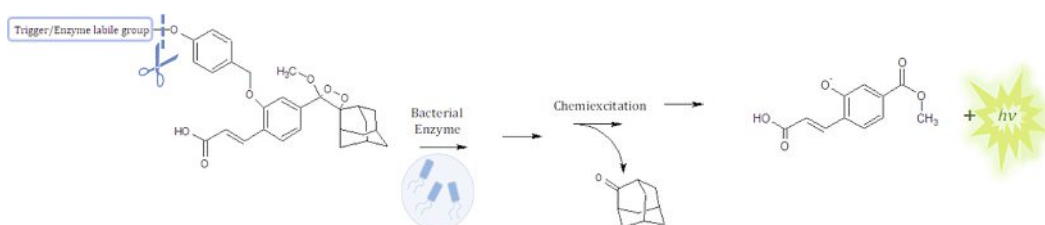
The current article presents the novel AquaSpark™ technology, used by NEMIS Technologies AG, as a solution for the on-site detection of foodborne pathogens in the production environment of food processing facilities. Sensitivity and specificity of this novel approach are achieved by combining selective enrichment media, containing bacteriophages, together with chemiluminescent probes for the rapid detection of live target bacteria.

### 1.1 AquaSpark™ Technology

Chemiluminescence is a light emitting process that occurs as a result of a chemical reaction.<sup>[15,16]</sup> In contrast to fluorescence, which requires an excitation light source, the energy needed for the excitation process of chemiluminescent materials lies within their molecular structure.<sup>[17,18]</sup> The light emission takes place due to the formation of a high-energy intermediate, which emits light upon returning to its ground state.<sup>[19]</sup> Generally, chemiluminescent compounds undergo chemical reactions, which are oxidation-dependent, in order to produce light. This makes them unsuitable for biologically relevant markers that are not oxidative in nature.<sup>[20]</sup> The discovery of a new chemiexcitation pathway, that is oxidation-independent, was made by Schaap and coworkers in 1982.<sup>[21]</sup> The chemiluminescence probes in this pathway were 1,2-dioxetanes (Schaap’s dioxetanes) with modifications at specific positions of their molecular structure.<sup>[21]</sup> These were found to be highly emissive probes, however, in aqueous solutions the light emission is relatively weak, which limits their use for applications in biology.<sup>[21]</sup> In order to enhance their light emission properties, Shabat’s research group in Israel recently found that, by modifying the chemical structure of Schaap’s dioxetanes, a maximum of 3’000-fold increase in chemiluminescent intensity was observed.<sup>[19]</sup> This discovery led to the development of AquaSpark™.

Aquasparks are a class of small-molecule chemiluminescent probes (phenoxy-dioxetane luminophores), that have been modified at certain positions in order to enhance their light emitting properties in aqueous solutions.<sup>[19]</sup> The discovery of these highly emitting chemiluminescent probes led to the design of reporter molecules for the detection of *Salmonella* and *L. monocytogenes* by Roth-Konforti *et al.*<sup>[9]</sup> These probes contain a phenoxy-dioxetane luminophore, carrying a triggering responsive group that serves as a substrate for a specific bacterial enzyme.<sup>[9]</sup> If the target bacteria are present, expressing the specific enzyme, the trigger will be cleaved, leading to a chemiluminescent reaction with the emission of light (Fig. 1). The luminescence can be measured as relative light units (RLU) and will indicate the presence of the specific target bacteria.

Fig. 1. Phenoxy-dioxetane luminophores designed for the detection of bacterial enzymes (AquaSpark™). The chemiexcitation pathway is initiated by the cleavage of a trigger responsive group due to the presence of a specific bacterial enzyme. The emission of light indicates the presence of the specific target bacteria. Modified from ref. [9]: M. Roth-Konforti, O. Green, M. Hupfeld, L. Fieseler, N. Heinrich, J. Ihssen, R. Vorberg, L. Wick, *Angew. Chem. Int. Ed.* **2019**, *58*, 10361.



## 1.2 Specificity of Enrichment Media

When developing a novel detection method, there is a need for a certain sensitivity and specificity when compared to the gold-standard reference methods.<sup>[22]</sup> Sensitivity, in this sense, refers to the proportion of actual positives, which are correctly identified. Specificity refers to the proportion of actual negatives that are correctly identified.<sup>[22]</sup> In other words, the culture medium or selective agar of choice should allow growth of the target bacteria but inhibit growth of any competing microflora that may lead to false negative (FN) or false positive (FP) results. Selective culture media are used to isolate specific bacteria based on their intrinsic resistance to particular inhibitory agents.<sup>[22]</sup> The base of the medium contains all of the necessary nutrients for bacterial growth together with the addition of antibiotics, chemicals, dyes or salts.<sup>[23]</sup> Bile salts, for example, are often used in selective media to control the growth of gram-positive bacteria, but allow growth of the usual gram-negative intestinal inhabitants.<sup>[23]</sup> Another well-known selective agent is the dye, Brilliant Green, used to inhibit gram-positive bacteria as well as some gram-negatives.<sup>[24,25]</sup>

A further advancement in the field of selective culture media was the addition of specific fluorogenic or chromogenic enzyme substrates to selective agar. These allow for the detection and enumeration of target bacteria based on the presence of fluorescence or a distinct color change due to the release of a chromogen.<sup>[26]</sup> These methods are currently the most widespread, however, chemiluminogenic assays for the detection of enzyme expression have been reported to be orders of magnitude more sensitive.<sup>[9,27–29]</sup> Roth-Konforti *et al.* show that the AquaSpark™ molecules, specific for the detection of *L. monocytogenes*, compared with the fluorogenic commercial analogue, 4-methylumbelliferyl-myo-inositol-1-phosphate, have an enhanced sensitivity of approximately 600-fold.<sup>[9]</sup> By using AquaSpark™ molecules we obtain increased sensitivity of our detection method compared with methods currently used, however, a certain level of specificity and selectivity is also required. Bacteria that are found on environmental surfaces are usually injured due to drying stress, in order to detect these bacteria after sampling, the enrichment culture needs to contain the necessary nutrients for their recovery, whilst at the same time control growth of non-target bacteria that may outcompete the target bacteria for nutrients.

The current, gold-standard methods for the detection of *L. monocytogenes* in food or on surfaces, requires a pre-enrichment

step before using chromogenic selective agar, which usually have higher concentrations of selective agents, to detect target bacteria. This process can take up to one week to obtain a definitive result.<sup>[10,11]</sup> Many selective agents, such as acriflavine and nalidixic acid, used in *L. monocytogenes* enrichment broth and selective agars, have been demonstrated to have adverse effects on the recovery and growth of injured bacteria, depending on the concentrations of these agents used.<sup>[31]</sup> To eliminate the need for pre-enrichment, a semi-selective enrichment broth with a low concentration of antibiotics as well as the addition of bacteriophages, as specific selective components, constitute a one-broth solution.

Bacteriophages are highly specific viruses that infect bacterial cells and use bacterial resources for their own reproduction. Phages are found ubiquitously in the environment wherever bacteria are present, for example in soil, water and other environmental areas.<sup>[32]</sup> Co-evolution of phages with their bacterial hosts has led to remarkable specificity with regards to the recognition and infection of their target host bacteria.<sup>[33]</sup> The application of strictly virulent phages ranges from diagnostics and biocontrol in foods, to prevention and treatment of diseases in humans and animals.<sup>[32–34]</sup> In this article, we show that the use of phages for the growth inhibition of competing microflora enhances the specificity of our enrichment broths.

## 2. Results

### 2.1 Novel Chemiluminescent Reporter Molecules for *Listeria monocytogenes* Exhibit High Signal Intensity

The AquaSpark™ probe used for the detection of *L. monocytogenes* is marked with a myo-inositol 1-phosphate group.<sup>[9]</sup> This trigger group is a known substrate for the virulence factor phosphatidylinositol-specific phospholipase C (PI-PLC) expressed by *L. monocytogenes*.<sup>[35,36]</sup> In Fig. 2A we show that *L. monocytogenes* is detected within 24 h, by using the specific chemiluminescent probe (AquaSpark™). In addition to *L. monocytogenes*, other bacterial cultures were tested and we show that, despite growth of these bacteria, they cannot be detected by using the specific probe, due to lack of expression of PI-PLC. To ensure the presence of growth in all cultures, the optical density (OD<sub>600</sub>) was measured prior to the addition of AquaSpark™ (Fig. 2B). Thus, we demonstrate that, through the use of a novel chemiluminescent reporter molecule specific for *L. monocytogenes*, we are able to detect these bacteria within 24 h.

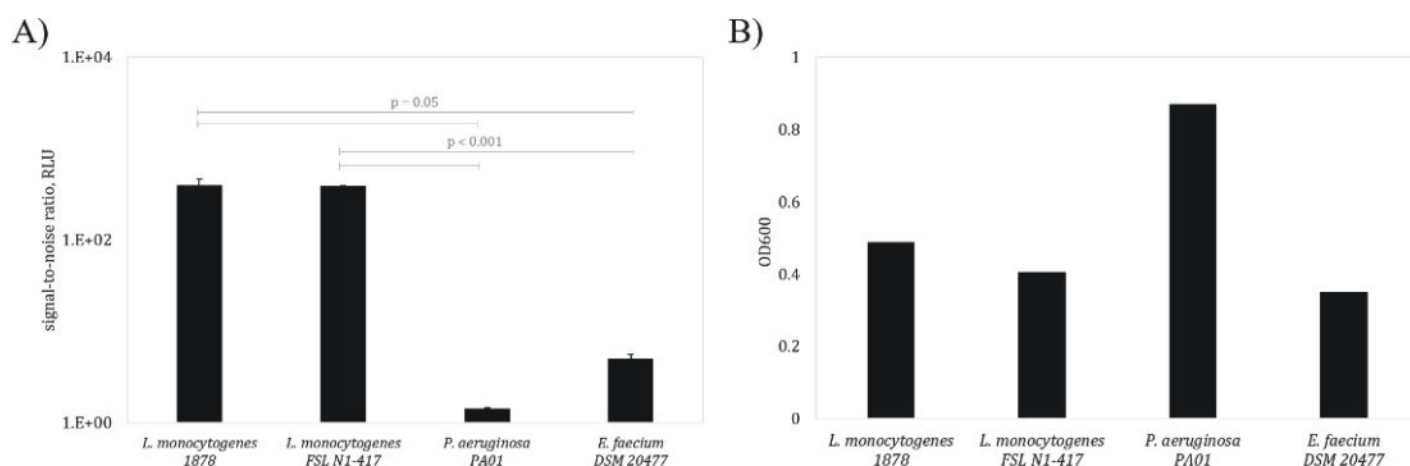


Fig. 2. Detection of *L. monocytogenes* using a chemiluminescent reporter molecule (AquaSpark™). A) Relative light units emitted by *L. monocytogenes* cultures based on the expression of PI-PLC. *L. monocytogenes* 1878 and FSL N1-417, *Pseudomonas aeruginosa* PA01 and *Enterococcus faecium* DSM 20477 were grown in enrichment media using an inoculum of 10<sup>2</sup> CFU/ml. After 24 h of incubation at 37 °C, AquaSpark™ was added to the cultures and the luminescence was measured. B) The optical density of each bacterial culture after 24 h incubation at 37 °C. Prior to the addition of AquaSpark™, the OD<sub>600</sub> of each bacterial culture was measured to ensure growth. The signal to noise ratios were calculated by dividing the value of luminescence in the bacterial cultures by the value of the background noise in the sterile control at t = 30 min. The values presented are an average of technical duplicates, with error bars indicating standard deviation. Statistical significance is represented by p-values, which were calculated by performing an unpaired t-test.

## 2.2 *Listeria monocytogenes* Can Be Detected on Stainless Steel Surfaces at Low Inoculation Levels

Fig. 3 illustrates an example of the specific detection of injured *L. monocytogenes* from a stainless steel surface within 24 h of sampling. To identify whether low levels of injured bacteria could be detected, stainless steel plates were inoculated with either  $10^2$  CFU/square or  $10^3$  CFU/square of *L. monocytogenes*. The surface was left to dry for 17 h, after which the bacteria were sampled and grown in an enrichment culture for 24 h at 37 °C. In addition to *L. monocytogenes*, separate surfaces were inoculated with  $\sim 1.5 \times 10^4$  CFU/square of *Enterococcus faecalis*. This is a common accompanying microbe, which does not express PI-PLC but disturbs the detection of *L. monocytogenes*. Fig. 3B depicts the signal to noise ratios of each sample with low ( $\sim 10^2$  CFU) or high ( $\sim 10^3$  CFU) levels of injured *L. monocytogenes*, as well as the sample containing *E. faecalis*, which does not emit any luminescence, merely at the level of background noise, due to the lack of PI-PLC expression. Therefore, the selective enrichment media used provides a suitable environment for the recovery of low numbers of injured bacteria from surfaces, which can then be specifically detected by the addition of the AquaSpark™ probe.

Until now, we have shown that healthy *L. monocytogenes* grown in a nutrient rich broth can be detected within 24 h using novel chemiluminescent reporter molecules (Fig. 2). Additionally, we have shown that injured *L. monocytogenes* can be recovered from a stainless steel surface at low inoculation levels and detected within 24 h (Fig. 3). In a factory setting, one needs to take into consideration that many other bacterial species will be present on surfaces, which may lead to the occurrence of FP or FN results in the detection method used. The virulence factor, PI-PLC, is not exclusively specific to *L. monocytogenes*. It has been found that both *L. ivanovii* and *Staphylococcus aureus* express PI-PLC.<sup>[37,38]</sup> Thus, FP results due to the presence of these non-target bacteria are possible. Furthermore, the presence of accompanying microbes, that do not necessarily express the specific enzyme, but outcompete with the target bacteria for nutrients, may lead to FN

results. This is the case with *E. faecalis*, which does not express PI-PLC (Fig. 3), however, is able to grow under the selective conditions used to enrich for *L. monocytogenes* and thus competes for nutrients.<sup>[39]</sup> To prevent the occurrence of either a FP or FN, an enrichment broth with an enhanced specificity for the target bacteria needs to be designed.

## 2.3 Bacteriophages Enhance the Specificity of Enrichment, Allowing Growth of *Listeria monocytogenes* without Competition for Nutrients by Accompanying Microbes

In Fig. 4 we show an example of the detection of *L. monocytogenes* and *L. ivanovii*, based on their expression of PI-PLC using the respective AquaSpark™ probe. PI-PLC expression by *L. ivanovii* is comparable to *L. monocytogenes* and, if present as microflora, would lead to a FP during the detection of *L. monocytogenes*. With the addition of specific phages, targeting *L. ivanovii*, to an enrichment broth, we show a significant decrease in signal-to-noise ratios by  $\sim 600$ -fold to a level of merely background noise. We also show that these phages have no significant effect on the signal produced by *L. monocytogenes* cultures, after the addition of AquaSpark™.

Therefore, we demonstrate the use of bacteriophages to target bacteria that could lead to FP results, such as *L. ivanovii*, due to their expression of the virulence factor PI-PLC. Furthermore, we would like to target bacteria that could lead to FN results, such as *E. faecalis*. As previously mentioned, this bacterium does not express PI-PLC but is able to grow in the same niche as *L. monocytogenes* and is thereby considered a competitor for nutrients and may outgrow *L. monocytogenes*. In order to detect *L. monocytogenes* in the presence of a potential competitor, stainless steel plates were inoculated with single and co-cultures of *L. monocytogenes* and *E. faecalis*. In Fig. 5B we show that, in the presence of *E. faecalis*, *L. monocytogenes* cannot be detected using AquaSpark™. However, when co-cultured bacteria are incubated in an enrichment broth containing a cocktail of phages targeting *E. faecalis*, we can efficiently detect *L. monocytogenes*.

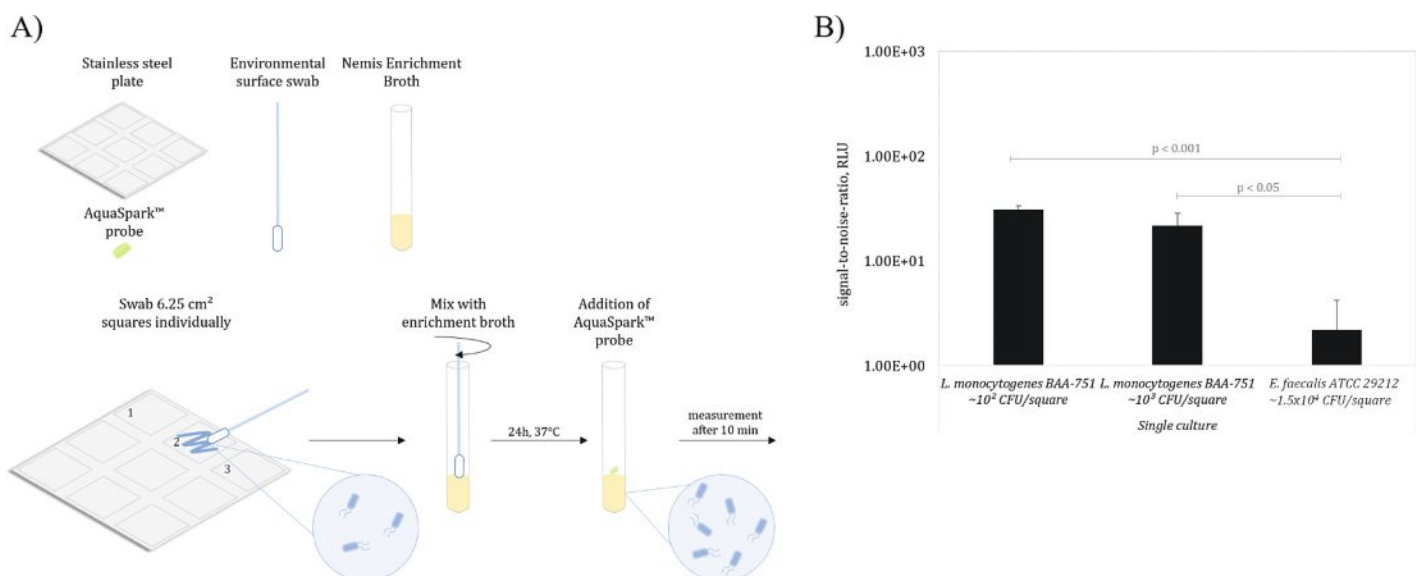


Fig. 3. Specific detection of low and high numbers of injured *L. monocytogenes* from a stainless steel surface using a chemiluminescent reporter molecule (AquaSpark™). A) Swabbing procedure: 6.25 cm<sup>2</sup> squares of a stainless steel plate are inoculated with low ( $\sim 10^2$  CFU/square) or high ( $\sim 10^3$  CFU/square) levels of *L. monocytogenes* BAA-751 or  $1.5 \times 10^4$  CFU/square of *E. faecalis* ATCC 29212, in technical triplicates. The plates are left to dry for 17 h at room temperature. Prior to sampling, swabs are pre-moistened, swabbed over the surface and the bacteria are recovered by mixing the swab with the enrichment broth. The tubes are incubated for 24 h at 37 °C and bacteria are detected 10 min after the addition of the AquaSpark™ probe by measuring luminescence. B) Detection of low and high inocula of *L. monocytogenes* on a stainless steel surface: The signal to noise ratios were calculated by dividing the value of luminescence in the bacterial cultures by the value of the background noise in the sterile control 10 min after addition of the AquaSpark™ probe. The results are depicted as average values between technical triplicates with error bars indicating standard deviation. Statistical significance is represented by p-values, which were calculated by performing an unpaired t-test.

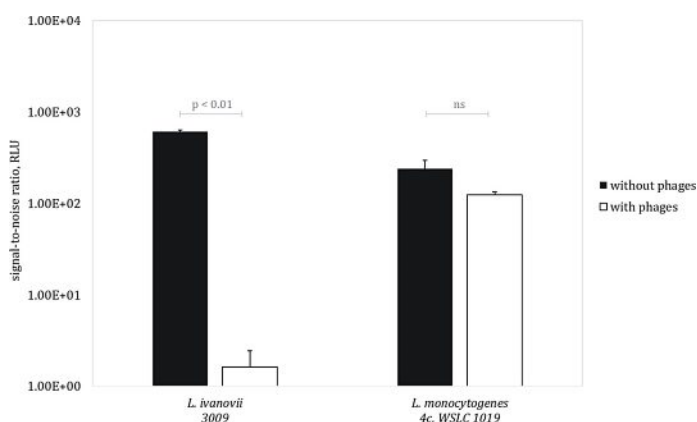


Fig. 4. *L. ivanovii* is efficiently targeted by phages without affecting *L. monocytogenes*. *L. ivanovii* 3009 and *L. monocytogenes* 4c, WSLC 1019 were grown in an enrichment broth without the addition of phages targeting *L. ivanovii* (black bars), using an initial inoculum of  $10^2$  CFU/ml, and with the addition of phages targeting *L. ivanovii* (white bars), using an initial inoculum of  $10^4$  CFU/ml, for 24 h at 37 °C. The signal to noise ratios were calculated by dividing the value of luminescence in the bacterial cultures by the value of the background noise in the sterile control 10 min after addition of the AquaSpark™ probe. The results are depicted as average values between technical triplicates with error bars indicating standard deviation. Statistical significance is represented by p-values, which were calculated by performing a paired t-test. ns = not significant

Therefore, we can conclude that injured *L. monocytogenes* that are co-cultured with *E. faecalis* and recovered from a surface, can be detected using AquaSpark™ by enriching the bacteria in a broth containing phages targeting *E. faecalis*.

### 3. Discussion and Outlook

In this study we have developed a new approach to specifically detect the common food-borne pathogen *L. monocytogenes* on surfaces of food manufacturing plants. Due to the ubiquitous nature and the environmental lifestyle of this bacterium,<sup>[40]</sup> it can be found on nearly every surface and is able to survive harsh conditions such as high salt concentrations, low refrigeration tem-

peratures and a range of pH values.<sup>[41–44]</sup> *L. monocytogenes* has been found to persist in food processing facilities over long periods of time.<sup>[45,46]</sup> In this context, persistence is defined as the continual presence of a clonal population of bacteria that can be found at a specific location over extended periods of time (from 6 months onwards).<sup>[46]</sup> It is thought that the formation of biofilms as well as resistance to sanitizers and other extrinsic stresses enables persistence, however, it is not fully understood and the basis of this is unknown.<sup>[47]</sup> Persistent strains of *L. monocytogenes* are strains that have been repeatedly isolated from the same location, this means that each production facility should be aware of the areas from which these strains have been isolated and focus on continuous monitoring in order to prevent cross-contamination of food. The significance of properly established environmental monitoring programs in food production facilities is proving to be of paramount importance in order to prevent the occurrence of foodborne outbreaks.

In order for monitoring programs to function correctly, extensive cleaning and disinfection strategies need to be implemented, followed by surface monitoring for contamination by making use of a rapid bacterial detection method.

Novel technologies for the detection of *L. monocytogenes* in food processing facilities should exhibit high sensitivity and specificity in order to obtain equivalent results compared to the standard reference method. This is mostly achieved by using a selective growth medium that prevents FP results while stimulating growth of the target bacterium, together with a distinct signal in the case of a positive result. The suitability of the AquaSpark™ molecules for this purpose was recently proven.<sup>[9]</sup> In this article we show that we have optimized the selectivity of the *L. monocytogenes* enrichment broth for the reliable, handy, quick and on-site environmental monitoring of *L. monocytogenes*. Accompanying microbes that interfere with the detection of *L. monocytogenes* are controlled, due to the specificity and efficacy of bacteriophages in liquid culture. As an example, we demonstrated the control of *E. faecalis* and *L. ivanovii* during detection of *L. monocytogenes*, thereby enhancing sensitivity and specificity of the assay. The method is currently undergoing international AOAC validation, which will deem it as comparable to the gold-standard reference

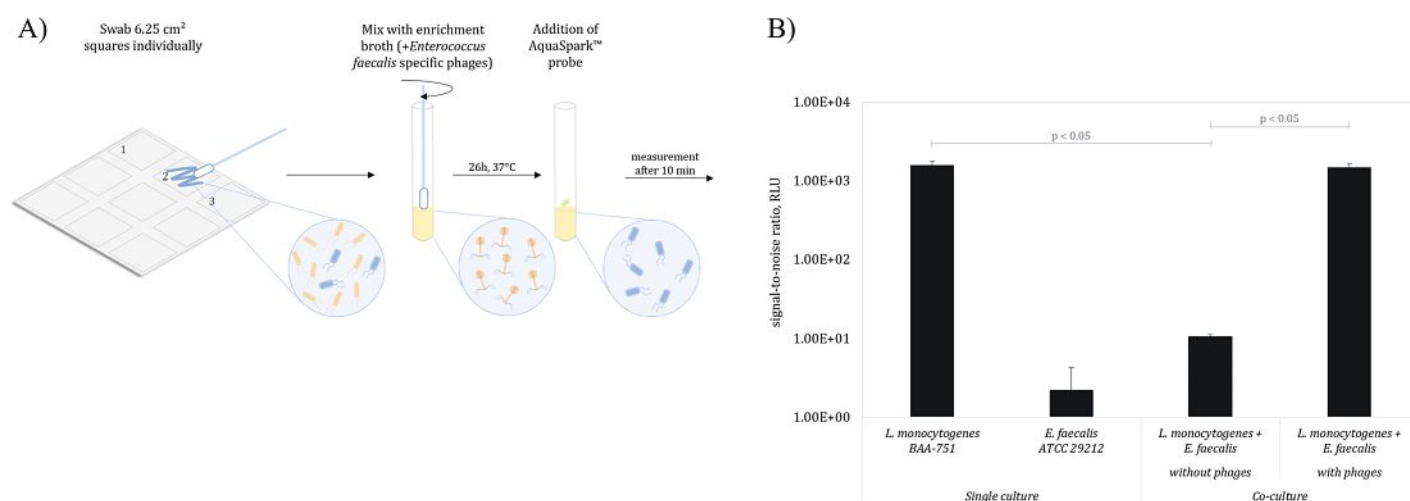



Fig. 5. Detection of *L. monocytogenes* from a stainless steel surface as a single or co-culture with *E. faecalis*. A) Swabbing procedure. 6.25 cm<sup>2</sup> squares of a stainless steel plate are inoculated with  $\sim 10^3$  CFU/square *L. monocytogenes* BAA-751 and  $\sim 10^4$  CFU/square *E. faecalis* ATCC 29212. Single- or co-cultures of both bacteria are spread over each square in technical triplicates. The plates are left to dry for 17 h at room temperature. Prior to sampling, swabs are pre-moistened, swabbed over the surface and the bacteria are recovered by mixing the swab with the enrichment broth with or without the addition of *E. faecalis* specific phages. The tubes are incubated for 26 h at 37 °C and bacteria are detected 10 min after the addition of the AquaSpark™ probe by measuring luminescence. B) Detection of *L. monocytogenes* and *E. faecalis* as single- or co-cultures on a stainless steel surface. The signal to noise ratios were calculated by dividing the value of luminescence in the bacterial cultures by the value of the background noise in the sterile control 10 min after addition of the AquaSpark™ probe. The results are depicted as average values between technical triplicates with error bars indicating standard deviation. Statistical significance is represented by p-values, which were calculated by performing an unpaired t-test.

method. It is very well suited for environmental monitoring, due to the possibility of on-site testing, without the need for laborious sample preparation or a fully equipped laboratory. Furthermore, a definitive result can be achieved within 24 h. In the future, we aim to further develop the platform technology, add a wide set of on-site detection methods for food manufacturers for the monitoring of additional pathogenic bacteria such as *Salmonella* and *Campylobacter* as well as hygienic indicators such as *E. coli* and coliform bacteria.

### Acknowledgements

The AquaSpark™ has been jointly developed together with the research group of Doron Shabat in Tel Aviv and the Swiss Biosynth AG. We would like to thank all persons involved in chemical synthesis and improvement of the AquaSpark™ molecules in many different ways, in particular Urs Spitz and Julian Ihssen.

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<b>NEMIS Technologies AG</b>	
NEMIS is a diagnostic startup based in the canton of Zurich. Its goal is the development and commercialization of microbial detection tests. The first focus of NEMIS is food safety and hygiene monitoring but its technology platform can also be applied in clinical diagnostics and animal health.	
<b>Key Figures</b>	
Founding Year	2018
Location(s)	Headquarter: Dübendorf
Legal Form	AG
Founders	Arnaud Muller Dr.sc. Mario Hupfeld Dr. Urs Breitenstein Roger Meier Biosynth AG Tel Aviv University
Scientific Advisors	Prof. Dr. Lars Fieseler Dr. Jeff Banks Prof. Adrian Egli Dr. Oliver Nolte
No of employees	6
<b>Key Inventions</b>	
AquaSpark	Chemiluminescent molecule class for the detection of microorganisms
Phage Technology	Selective enrichment by tailored phage cocktails for specific detection
<b>Next Milestones</b>	
September 2020	AOAC certification 1 <sup>st</sup> product
November 2020	Product Launch 1 <sup>st</sup> product
December 2021	3 products on the market
<b>Awards</b>	
November 2019	<b>Finalist Swiss Technology Award</b>
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