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<b>General information</b>	
PPP number	<b>TKI-AF-16141</b>
Title	<b>Rapid at-line detection of environmental <i>Listeria</i> in the food industry</b>
Roadmap/Umbrella	<b>TKI AF Kernthema Gezond en Veilig</b>
Executive knowledge institution(s)	<b>WFBR</b>
Research project leader (name + e-mail address)	<b>Heleen van den Bosch Heleen.vandenbosch@wur.nl</b>
Coordinator (on behalf of private parties)	<b>Gerold de Valk (BiosparQ) devalk@biosparq.nl</b>
Government contact person	<b>M.G.M. van Creij</b>
Start date	<b>01-01-2017</b>
End date	<b>31-12-2019</b>

<b>Approval coordinator/consortium</b>	
The coordinator has assessed the annual report on behalf of the consortium:	<input checked="" type="checkbox"/> approved <input type="checkbox"/> rejected
Possible feedback on the annual report:	

<b>Short content description/aim PPS</b>
<p>Suitable diagnostic tests for rapid, at-line detection, identification and typing of microorganisms are hardly available to the food industry. In this project BiosparQ's technology that is based on 'single cell' analysis of bacteria by means of MALDI TOF MS will be used for the detection of microorganisms. The ultimate goal is the assessment of the full bacterial composition of a sample in a couple of minutes. Enrichment and culturing of samples would not be necessary, since each cell is detected and identified and typified separately.</p> <p>The primary focus in the project will be on the detection of environmental <i>Listeria</i> which is a serious risk in food processing plants.</p> <p>A successful introduction of the technology would require the development of rapid and dedicated sample pretreatment protocols to concentrate sufficient microorganisms to a small volume. The participants will explore new ways to concentrate bacterial cells from food products.</p> <p><u>In global lines the project will deliver the following products/results:</u></p> <ul style="list-style-type: none"> <li>• Specific sample pretreatment protocols for environmental <i>Listeria</i> swabs and for a number of</li> </ul>

food samples to be chosen by the participating food industries. These protocols will be focused on the concentration of the population of bacterial cells from swabs or food samples to a small volume (50 to 100 µL) that will be used for subsequent analysis by single cell MALDI TOF MS.

- A single cell MALDI TOF MS apparatus (BiosparQ) that is suited for the rapid characterisation (some minutes) and typing of populations of bacterial cells.
- MALDI TOF MS database information specific for the typing of individual bacterial cells such as *Listeria (monocytogenes)* and other pathogenic and food spoilage microorganisms as chosen by the participating food companies.
- Validated procedures to detect *Listeria (monocytogenes)* in environmental samples by BiosparQ technology.
- Validated procedures to detect populations of pathogenic and/or spoilage microorganisms by BiosparQ technology.

### Planning and progress

Is the PPP going according to plan? <sup>1</sup>	In the project plan, it was described that the development of sample pretreatment protocols would be started in the first year. It was proposed to work with stacks of nitrocellulose membranes with decreasing nominal pore sizes. However, both Nestlé and Cargill proposed in the kick-off meeting to look for other techniques than filtration to concentrate cells in a small volume. Filtration has been investigated for many years, but until now no breakthrough procedure was achieved with this technique. They asked the consortium to look for the “difficult way”, and to come up with new, innovative solutions. Therefore the first year was used to extensively search for other alternative pretreatment methods. Out of several proposals, two methods have been chosen to start with in 2018.
Have there been changes in the consortium/project partners?	Yes, several new partners have been found (see highlights).
Is there a delay and/or deferred delivery date?	Due to reasons outlined above there was a delay with the start of the practical work, and, therefore, the delivery date of Milestone 1 has been postponed.
Are there any substantive bottlenecks? Provide a brief description	No
Are there any deviations from the projected budget?	No
Do you expect a patent application to arise from this PPP?	This may be the case if the new sample pretreatment procedures will be shown to be successful.

<sup>1</sup> If applicable, use the explanation from the financial project report

Current summary of the project for the website Kennisonline

Suitable diagnostic tests for rapid, at-line detection, identification and typing of microorganisms are hardly available to the food industry. Therefore, quick adaptation to the microbial status of half-products and during processing steps is very difficult. The use of at-line diagnostic tests for microorganisms would enable a next step in processing within 1 to 2 hours and at least on the same day. Such a processing optimisation may lead to a considerable reduction in costs and will also have direct effects on food safety, food spoilage and sustainability.

BiosparQ is developing an innovative technology that is based on 'single cell' analysis of bacteria by means of MALDI TOF MS. The ultimate goal is the assessment of the full bacterial composition of a sample in a couple of minutes. Enrichment and culturing of samples would not be necessary, since each cell is detected and identified and typified separately. A successful introduction of the technology would require the development of rapid and dedicated sample pretreatment protocols to concentrate sufficient microorganisms in the test volume. The participants will explore new ways to concentrate bacterial cells from food products into small volumes. In addition to electro-adsorption technology and stacks of nitrocellulose membranes with decreasing nominal pore sizes, specific focus will be on the development of metal membranes that are based on a new and patented technology enabling the production of membrane filters with unique characteristics. The food industry sector will broadly profit from this project. The primary focus will be on the detection of environmental *Listeria* which is a serious risk at food processing plants. In addition, detection procedures will be developed for other microbial populations as well. The innovative developments in the project will be valuable to the scientific community, since the sample pretreatment procedures and the BiosparQ technology will be applicable in many other sectors as well; the project will boost scientific developments in other sectors. The society will profit from a substantially better detection procedure for environmental *Listeria*, resulting in less food products being contaminated with *Listeria monocytogenes*.

**Highlights:**

- Several new partners have been found:
  - Arla Foods
  - Diversey
  - World Bioproducts
  - Mecon Engineering BV (already in project proposal, but did not sign the consortium agreement because the company was taken over at that time.)
- A new smaller prototype of the Biosparq's single cell MALDI TOF MS was built by Mecon. This is a step forward to the desired machine's miniaturization.
- The first year was used to extensively search for appropriate pretreatment methods. Out of several proposals, two methods with "functionalized", paramagnetic beads have been chosen to start with this year:
  - Coating with synthetic peptide domains that bind microorganisms. These domains can interact with the peptidoglycan layer of gram-positive bacteria. The domains will be cloned and expressed in *Pichia pastoris*.
  - Coating with lectins. Lectins are carbohydrate-binding proteins. Individual protein-carbohydrate interactions are usually weak, therefore multi-valency is often required to achieve relevant binding affinities. In this project BioMag®-Plus, superparamagnetic microparticles will be used.

**Number of delivered products in 2016**

Academic articles	Reports	Articles in journals	Introductions/workshops
x	x	x	x

**Appendix: Names of the products or a link to the products on a public website**

**Link naar Kennisonline/TKI AF:**

<https://www.wur.nl/en/Research-Results/kennisonline/AF16141-Rapid-at-line-detection-of-environmental-Listeria.htm>

<http://topsectoragrifood.nl/project/rapid-at-line-detection-of-environmental-listeria-in-the-food-industry/>

Akkoord: Hans van der Kolk (Topsectorsecretaris)