Validation of Innovative Tools to Assess and to Improve Microbiological Safety in the Food Chain

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**Presenters:**  
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*PepsiCo, United Kingdom*

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Today’s Presenters

Pedro J. López is Physicist and MSc on Data Science with research background. Expert in complex analysis and AI applications’ building, he is responsible of making value out of data at Artificial Intelligence Talentum. His work is focused on advanced statistical analysis, data mining, and data visualization techniques, to create solutions that enable enhanced business performance.

Pietro Cattaneo, PhD in Life Sciences – University of Lausanne, Switzerland - M.Sc. in Cell Molecular Biology - University of Milano, Italy.
He contributed deciphering the molecular mechanisms of plant development by identifying and describing two genes involved in plant root growth.
He stepped into the biotech food sector joining the start-up SwissDeCode SA in 2018. He now holds many simultaneously roles such as developing new rapid DNA detection solutions, running wet-lab activities, interacting with customers during the pre- and post-sales process, and leading product demonstrations.

Dr. Trevor Phister received his PhD in Food Microbiology from the University of Minnesota in 2001. He held a number of academic positions in both the US and the UK before joining PepsiCo in 2013. He is currently a Principal Microbiologist in the Global Microbiology team based in Europe and a Co-Chair of the PepsiCo Global Microbiology Council. In his current role, he works with teams to develop and maintain microbiology programs ranging from the assessment of new microbial methods to the development of policies and tools to support risk assessment of materials, products and processes across the PepsiCo portfolio.
WEBINAR

Validation of Innovative Tools to Assess and to Improve Microbiological Safety in the Food Chain

June 23, 2020

Luca Cocolin, Università di Torino
What is the EIT?
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- 252 research centres
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We are on a MISSION

EIT Food is an ecosystem for solving complex societal challenges by deploying innovative solutions

EIT Food is the largest European consumer-centric open science innovation ecosystem
VITAL concept

Food must be safe for the consumer (pre-requisite) but microbiological analysis are time consuming and unfit for the modern context. Rapid methods can be a possibility, but they need to be validated and validation costs a lot of money for food producing companies.

Proposed solution: Combine digitalisation and new sensor technologies to steer and improve efficiency in the food value chain.

VITAL ambition and impact

Assure food safety based on the smart exploitation of two innovative tools: artificial intelligence (AI) and lab-on-chip sensors, respectively. AI will be used to analyse available data provide a prediction tool that will allow a more effective validation scheme of rapid methods in an industrial setting.

It is expected that due to the lower demand requested for the validation of new rapid methods, those possibilities will be more often employed at industrial level resulting in better assessment of foodborne pathogens in the food chain and safer foods for the consumers.
VITAL Project: The AI-Approach

PEDRO JESÚS LÓPEZ ABENZA
MSc Data Scientist at AI Talentum

Email: plopez@aitalentum.com
# Table of Contents

1. Introduction: Who are we?
   - What is AI Talentum?

2. VITAL Project: The AI approach
   - Digitalization Process
   - The AI role
   - VITAL Project Scheme

3. An overview about AI solutions
   - Introduction to AI solutions
   - Typical AI techniques
   - Other food-related project: Automated inspection of caps
   - AI in Biological Industry and Food Manufacturing

4. Conclusions

5. Q&A
Introduction: Who we are?

- AI start-up located in the south of Spain
- Experts on ITC solutions and Industry 4.0
- Partner of the EIT Food Consortium
- VITAL: Data Science Department
VITAL Project: Digitalization Process

- Redefinition of tasks in order obtain better performance by digitalizing actions and new technologies.

- Digitalization technologies encompass a series of digital solutions, such as:
  1. Artificial Intelligence (AI)
  2. Cloud Infrastructure
  3. Internet of Things (IoT)
  4. Others (Blockchain & Cybersecurity)

- Identifying weaknesses from well-established processes and coping with new approaches capable of enhancing its performance
VITAL Project: The AI Role

- New procedure for the rapid methods validation.
- Automated calculus for an optimized validation procedure for the analyzed method
- Reduce the numbers of samples to be tested, allowing to securely speed up the production
VITAL Project: Scheme
Introduction to AI solutions

- AI systems use data in order to understand complex processes and learn about behavior and responses

- These solutions are based in a wide variety of techniques from Machine Learning, DL, NLP or even Computer Vision.

- The AI

\[
\{x\}: \text{Input variables} \quad \xrightarrow{\text{AI BLACK BOX}} \quad f(\{x\}): \text{Output function}
\]
**Introduction to AI solutions**

- Data contain parametrized information about processes. They may come from many sources and have different structures:
  1. Tabular data
  2. Unstructured data
  3. Images
  4. Raw text

- AI systems learn behavior from complex processes and their parametrized input-attributes.

- Models are capable of predicting values by new incoming data from these processes.

- Datatypes:
  1. Categorical: YES/NO, HIGH/MED/LOW
  2. Numerical: 1.6, 2.22, 1074…
Typical AI techniques: An overview
Typical AI techniques: Classification

- **Supervised Learning**: Prediction of the value of an objective attribute using IA techniques that learn from the training data.

  1. **Classification**: Categorical prediction
  2. **Regression**: Numerical prediction

---

### Some techniques:
- Naïve Bayes
- Decision Tree
- Logistic Regression
- SGD
- Support Vector Machines
- Neural Network
- Ensembles

---

### Example Table

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<td>160.94.179.252</td>
<td>139</td>
<td>172</td>
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<td>206.163.37.95</td>
<td>11:14:38</td>
<td>160.94.179.251</td>
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<td>285</td>
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<td>11:14:41</td>
<td>160.94.179.250</td>
<td>139</td>
<td>195</td>
<td>No</td>
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<tr>
<td>10</td>
<td>206.163.37.95</td>
<td>11:14:44</td>
<td>160.94.179.249</td>
<td>139</td>
<td>163</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Typical AI techniques: Clustering

- **Clustering**: Group cases based on the similarity of their data attributes.
  Three main approaches:

  1. Partitional clustering
  2. Hierarchical clustering
  3. Density-based clustering
Typical AI techniques: Deep Learning
Other food-related project: Automated inspection of caps

Tasks:

1. Intelligent assistant for an already implemented CV systems for the inspection a process of production of caps

2. Analysis of possible errors on the detection of wrong caps by different profiles analyzed by the vision system

3. Optimization of the number of human inspection on the caps production line

Tools: Classification techniques

1. Logistic regression
2. Random Forest
AI in Food Manufacturing

- Manufacturing industry is being revolutionized by technology.

- Nevertheless, it has meant **new challenges** in the industry:

  1. Sustainable adoption of advanced manufacturing technologies
  2. Agile and flexible enterprise capabilities and supply chains
  3. Close collaboration between industry and research to adopt new technologies.
AI examples in Biological Industry

- Examples of applications:

1. AI system for reducing the animal toxicity tests in consumer products (*CAAT* from *Johns Hopkins University*)

2. AI sensors for detecting foodborne pathogens at home (*IBM*)

3. AI system for reducing the pathogens in processing plants (*Luminous Group*)
Conclusions

- Understanding the role of the AI in VITAL project
- Presenting the motivation to using AI systems
- Introducing the main tools used by an AI system
- Exposing some applications of AI in biological environments
Thank you very much for your attention!

Time for your questions!
Foodborne Illness

What is Foodborne Illness?
Foodborne illness is a common, costly, sometimes life-threatening public health problem.

How is it caused?
Outbreaks and individual cases of foodborne illness result from consuming the two most common types of foodborne pathogens:

• Bacteria, like *Salmonella*, *E. coli O157:H7*, *B. cereus*, *S. aureus*, and *L. monocytogenes*
• Viruses, such as *norovirus* or *hepatitis A*

Who is at risk?
Anyone can get a foodborne illness. People such as pregnant women, young children, older people, and those with weak immune systems are more susceptible and at risk.
The US Centers for Disease Control and Prevention (CDC) estimates 48 million people are affected by foodborne illness annually, 128,000 people are hospitalized and 3,000 die.

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Food</th>
<th>Number of Outbreaks</th>
<th>Number of Outbreaks - Associated Illnesses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scombroid toxin (histamine)</td>
<td>Fish</td>
<td>12</td>
<td>35</td>
</tr>
<tr>
<td>Ciguatoxin</td>
<td>Fish</td>
<td>11</td>
<td>33</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Chicken</td>
<td>8</td>
<td>307</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>Dairy (unpasteurized)</td>
<td>7</td>
<td>57</td>
</tr>
<tr>
<td>Norovirus</td>
<td>Mollusk</td>
<td>6</td>
<td>209</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Pork</td>
<td>6</td>
<td>96</td>
</tr>
<tr>
<td>Vibrio parahaemolyticus</td>
<td>Mollusk</td>
<td>6</td>
<td>19</td>
</tr>
</tbody>
</table>
Foodborne Illness is Largely Preventable

At Home

• Practicing safe food handling
• Keeping your refrigerator clean and at 4º C/40º F, separating raw foods from cooked foods
• Cooking meats thoroughly
• Avoiding unpasteurized food products such as milk and cheese

Along the Food Supply Chain

• Following appropriate cleaning, manufacturing procedures, and adopting decontamination solutions
• Monitoring temperature and other important food conditions correctly
• Ensuring safe packaging, food warehousing, transportation, and inspection/QA practices
Foodborne diseases, caused by pathogenic bacteria, have become an important social issue in the field of food safety. True incidence of foodborne outbreaks is highly underestimated.

It is urgent to detect foodborne pathogens in order to control foodborne pathogen spread and reduce foodborne disease occurrence as well as economic burden.

Classical methods
New technologies for rapid detection of foodborne pathogens
Classical Methods: The “Gold Standards”

The conventional methods implemented in food analysis consist of sample homogenization and subsequent culturing of the microorganisms on agar plates followed by biochemical identification.

Vidic et al., 2019; Law et al., 2015.
## Classical Methods: The “Gold Standards”

<table>
<thead>
<tr>
<th>Disadvantages for the Food Industry Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Workflow</strong></td>
</tr>
<tr>
<td>• Laborious: pre-enrichment, selective enrichment, selecting plating, isolation and identification</td>
</tr>
<tr>
<td><strong>Timing</strong></td>
</tr>
<tr>
<td>• 2-3 days from sample preparation to result interpretation</td>
</tr>
<tr>
<td><strong>Lab equipment/Facilities</strong></td>
</tr>
<tr>
<td>• High demand of consumables such as Petri dishes and media</td>
</tr>
<tr>
<td><strong>Operators</strong></td>
</tr>
<tr>
<td>• Professional and trained personnel</td>
</tr>
</tbody>
</table>

Culture and colony-counting methods are inadequate for rapid detection of foodborne pathogens, especially for reduce foodborne disease occurrence in food industry.

Vidic et al., 2019; Law et al., 2015.
New technologies for Rapid Detection of Foodborne Pathogens

The growing amount of street foods and the increasing demand of ready-to-eat foods prompted the development of advanced, sensitive, specific and labor-saving detection methods that can identify pathogens accurately and rapidly in a timely manner.

Rapid detection methods can be categorized into:

1. Biosensor-based
2. Immunological-based
3. Nucleic acid-based
• Analytical device that consists of two main elements:
  1. The bioreceptor responsible for recognizing the target analyte can either be antibodies, nucleic acids, biological derived as well as synthetic polymers.
  2. The transducer that converts the biological interactions into a measurable electrical signal can be optical, electrochemical or mass based.
• Biosensors do not require sample pre-enrichment.

Vidic et al., 2019; Law et al., 2015.
### Biosensor-based: Advantages vs. Disadvantages

<table>
<thead>
<tr>
<th>Method</th>
<th>Assay-time</th>
<th>Detection Limit</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optical Biosensors</td>
<td>Not stated</td>
<td>E. coli O157:H7</td>
<td>• Sensitive</td>
<td>• Costly</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.4 $10^4$ CFU/mL</td>
<td>• <strong>Real time detection</strong></td>
<td>• Sensor calibration</td>
</tr>
<tr>
<td>Electrochemical biosensors</td>
<td>15 min</td>
<td>E. coli O157:H7</td>
<td>• <strong>High-throughput</strong></td>
<td>• Low specificity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.6 $10^1$ – 7.23 $10^7$ Cells/mL</td>
<td>• Automated</td>
<td>• <strong>Food matrices interference</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Laborious</td>
</tr>
<tr>
<td>Mass-based biosensors</td>
<td>4 h</td>
<td>E. coli O157:H7</td>
<td>• Cost effective</td>
<td>• Low specificity and sensitivity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 - 50 Cells/mL</td>
<td>• Easy</td>
<td>• <strong>Laborious</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Real-time detection</td>
<td>• Instrumentation design</td>
</tr>
</tbody>
</table>

Wang et al., 2013b; Shen et al., 2011; Varshney et al., 2005.
• Interaction antibody-antigen, whereby a particular antibody will bind to its specific antigen.
• The binding strength of a particular antibody to its antigen determines the sensitivity and specificity.

Vidic et al., 2019; Law et al., 2015.
## Immunological-based: Advantages vs. Disadvantages

<table>
<thead>
<tr>
<th>Method</th>
<th>Assay-time</th>
<th>Detection Limit</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>3 h</td>
<td>E. coli O157:H7 6.8 $10^3$ CFU/mL</td>
<td>• Specific</td>
<td>• Low sensitive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• <strong>High-throughput and automatized</strong></td>
<td>• False negatives</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Detection of bacterial toxins</td>
<td>• <strong>Cross-reactivity</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Trained personnel</td>
</tr>
<tr>
<td>Lateral flow immunoassay</td>
<td>10 h</td>
<td>Salmonella Typhi $10^4$-$10^5$ CFU/mL</td>
<td>• <strong>Cost effective</strong></td>
<td>• Labelling of antibodies or antigens required</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Easy to operate</td>
<td>• <strong>False positive</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Sensitive</td>
<td>• Specificity</td>
</tr>
</tbody>
</table>
• Detection of DNA or RNA specific sequences in the target pathogen.
• Hybridization between the target nucleic acid sequence and a synthetic oligonucleotide which is complementary to the target sequence.
• Detection of specific genes such as toxin-related genes, prevents ambiguity and wrong result interpretation.

Vidic et al., 2019; Law et al., 2015.
## Nucleic acid-based: Advantages vs. Disadvantages

<table>
<thead>
<tr>
<th>Method</th>
<th>Assay-time</th>
<th>Detection Limit</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
</table>
| Multiplex polymerase chain reaction – mPCR                            | 24 h       | Salmonella spp. 10^3 CFU/mL | • Detection of several pathogens at a time  
• Less costly  
• Detection of groups of pathogens | • Post-PCR result visualization  
• Skilled technical personnel  
• Required reaction conditions optimization |
| Real-time or quantitative PCR - qPCR                                   | <30 h      | Salmonella spp. 5 CFU/25g | • Amplification can be monitored at real time  
• Quantitative assay  
• Amplification product confirmed by melting curve | • Skilled technical personnel  
• Difficult in multiplex assay  
• False-positive results  
• High cost |
| Loop mediated isothermal amplification – LAMP                        | <20 h      | Salmonella spp. 5 CFU/10 mL | • High specificity  
• Isothermal conditions with great efficiency  
• Tolerance to biological inhibitors  
• Rapid | • Complex primer design  
• Required multiplex reaction optimization |

Ruiz-Rueda et al., 2011; Shao et al., 2011; Silva et al., 2011
SwissDeCode - SDC

- SwissDeCode is a Swiss based start-up founded in 2016.

- SDC helps farmers and food manufacturers to grow and produce food that is safe to eat.

- SDC develops and provide rapid DNA detection solutions applied to:

<table>
<thead>
<tr>
<th>Food Safety</th>
<th>Salmonella spp.</th>
<th>L. monocytogenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food Adulteration - Contamination</td>
<td>Porcine DNA</td>
<td>Vegetal DNA</td>
</tr>
<tr>
<td>Food Authenticity</td>
<td>Cheese AOC</td>
<td>A1 Milk</td>
</tr>
</tbody>
</table>
SwissDeCode into the VITAL Project

- SwissDeCode is developing a Lab on chip Solution - BEAMitup™ - to empower the quality control processes along the entire food supply chain, from farm to fork.

- SwissDeCode BEAMitup™ solution advantages:
  - Sensitivity
  - Specificity
  - Robustness
  - Cost
  - Labor-saving and simplicity
  - High-throughput
  - On-site detection
Take Home Message

• There is no a universal method for foodborne pathogens detection.

• Methods comparison and choice are based on:
  
  - Sensitivity and Accuracy
  - Specificity
  - Robustness
  - Cost
  - Labor-saving
  - Choice of matrix/Organism
  - Target foodborne pathogen
  - On-site detection
Impact of Recalls: Time to Act

- Number of food recalls between 2012 and 2017 in the US: +300%
- The average direct cost of a food recall: $10 M
- People who would choose the same brands following a recall: -45%

Arthur D. Little
Open Questions

• What can be implemented in rapid detection methods for foodborne pathogens?
• Which are the key requirements demanded by the Food Industry?
• How do we validate a new rapid detection method?
Pietro Cattaneo, PhD – Field Application/Product Development Scientist

Email: pietro.cattaneo@swissdecode.com

Mobile: +41 78 235 52 35
Questions?

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