Innovative Solutions for *Listeria* Detection and Monitoring

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Listeria

- **Challenges are increasing:**
  - **Increase in susceptible persons with aging population**
  - **Increase in the amount of ready-to-eat foods**
  - **Increase in demand for organic foods**
- **The economic impact of *Listeria* is enormous:**
  - **In terms of human health (medical costs, loss of life)**
  - **Significant number of foods have been involved in international product recalls**
  - **Some manufacturers have suffered huge financial losses through recall, extra freight, product disposal, product reworking etc.**

Solutions for Food Industry

- **The challenge for the food industry is to develop, implement, maintain and improve programs for *Listeria* monitoring and control**
- **The reference methods for *Listeria* in the EU are EN/ISO 11290-1 and EN/ISO 11290-2. The classical cultural methods for *Listeria* are time-intensive and laborious (4-7 days)**

Solutions:

- **Rapid analysis methods**
- **Reliable results to prove the absence of *Listeria***
- **Fast confirmation of microbiological test results**
- **Long-term surveillance and outbreak studies**

Solutions for Food Industry

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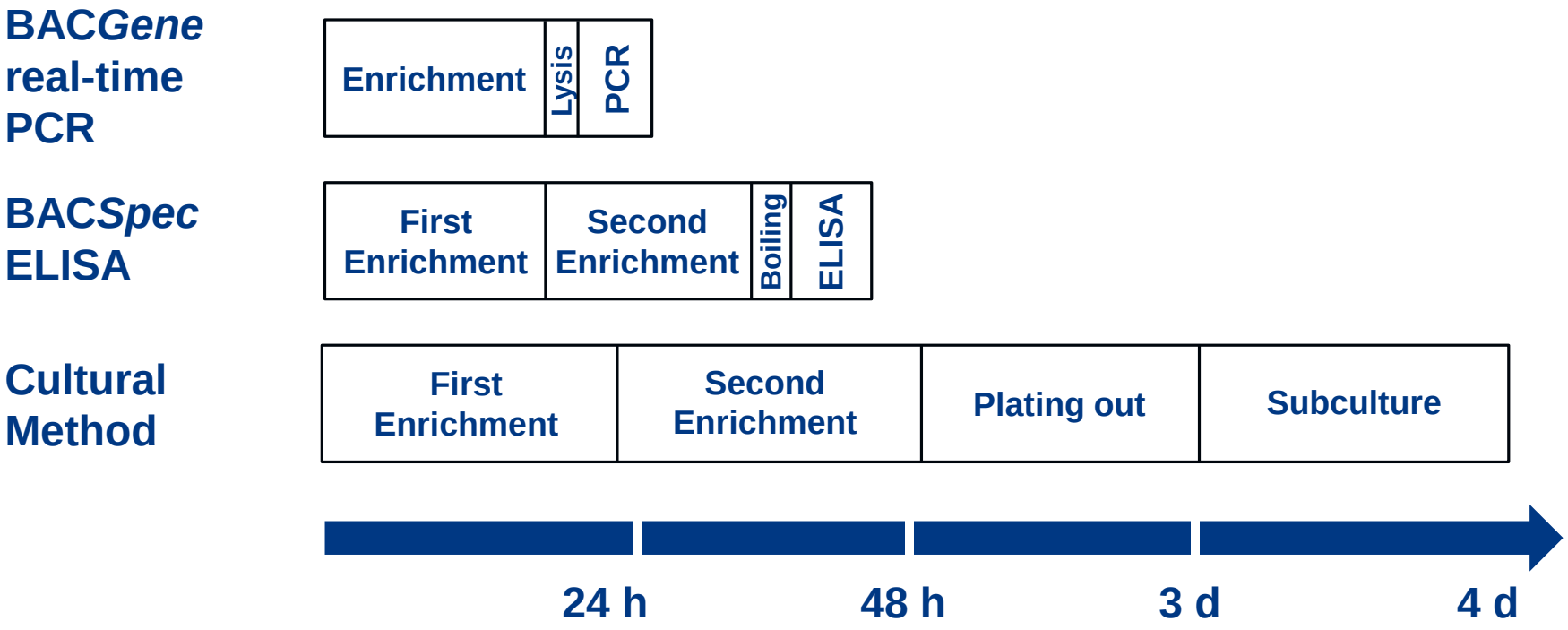
Time to Negative Results

Compared with conventional methods which can take several days, negative results are available with BAC*Gene* in less than 24 hours and with BAC*Spec* in less than two days.



Solutions for Food Industry

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BAC*Gene Listeria* Kits

- **BAC*Gene Listeria* Multiplex (AFNOR certified)**
  - *Listeria* spp.
  - *Listeria monocytogenes*
- **BAC*Gene Listeria* spp. (AFNOR certified)**
- **BAC*Gene Listeria monocytogenes* (AFNOR certified)**
- **AOAC certification in preparation**

BY AFNOR CERTIFICATION
NF VALIDATION EN ISO 16140

EGS 38/01-03/15
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
www.afnor-validation.com

BAC*Gene Listeria* Multiplex

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2-in-1 detection of *Listeria monocytogenes* and *Listeria* spp. in one well improves *Listeria* testing!

Improving Time to Results

- No secondary enrichment step necessary
- Simple lysis protocol

Minimizing Risk

- Probe-based real-time PCR assay to guarantee excellent specificity
- Positive and Negative PCR controls to demonstrate PCR target detection and absence of contamination
- Internal Positive Control (IPC) for verification of PCR performance and confirmation of results

Increasing Reliability

- High quality kit with AFNOR Certification
- Automated data analysis based on validated evaluation algorithm

Overview: BAC*Gene Listeria*

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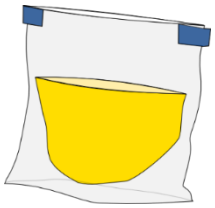
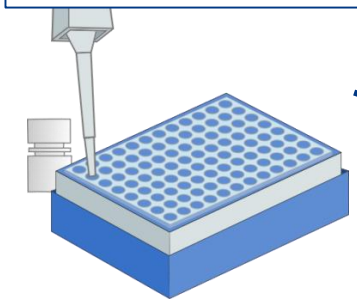
Enrichment in Actero™ Listeria Enrichment Media

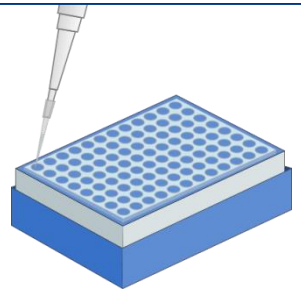


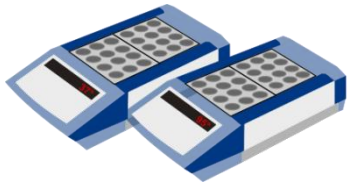
Transfer aliquot to sterile container

Prepare lysis buffer and dispense

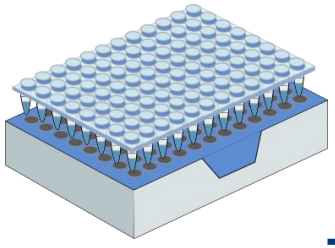


Transfer enrichment to lysis buffer

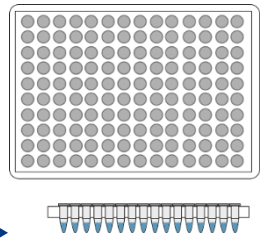




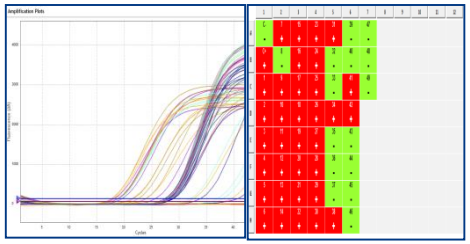
Thermal Lysis
- 37°C, 20 min
- 95°C, 10 min



Cooling & brief spin down



Transfer to PCR plate



PCR Run & Evaluation

Solutions for Food Industry

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Confirmation of Presumptive Positives

Germ identification = Confirmation of microbiological test results

Traditional methods

- Microscopy
- Gram coloring
- Biochemical Galleries
- Antibodies

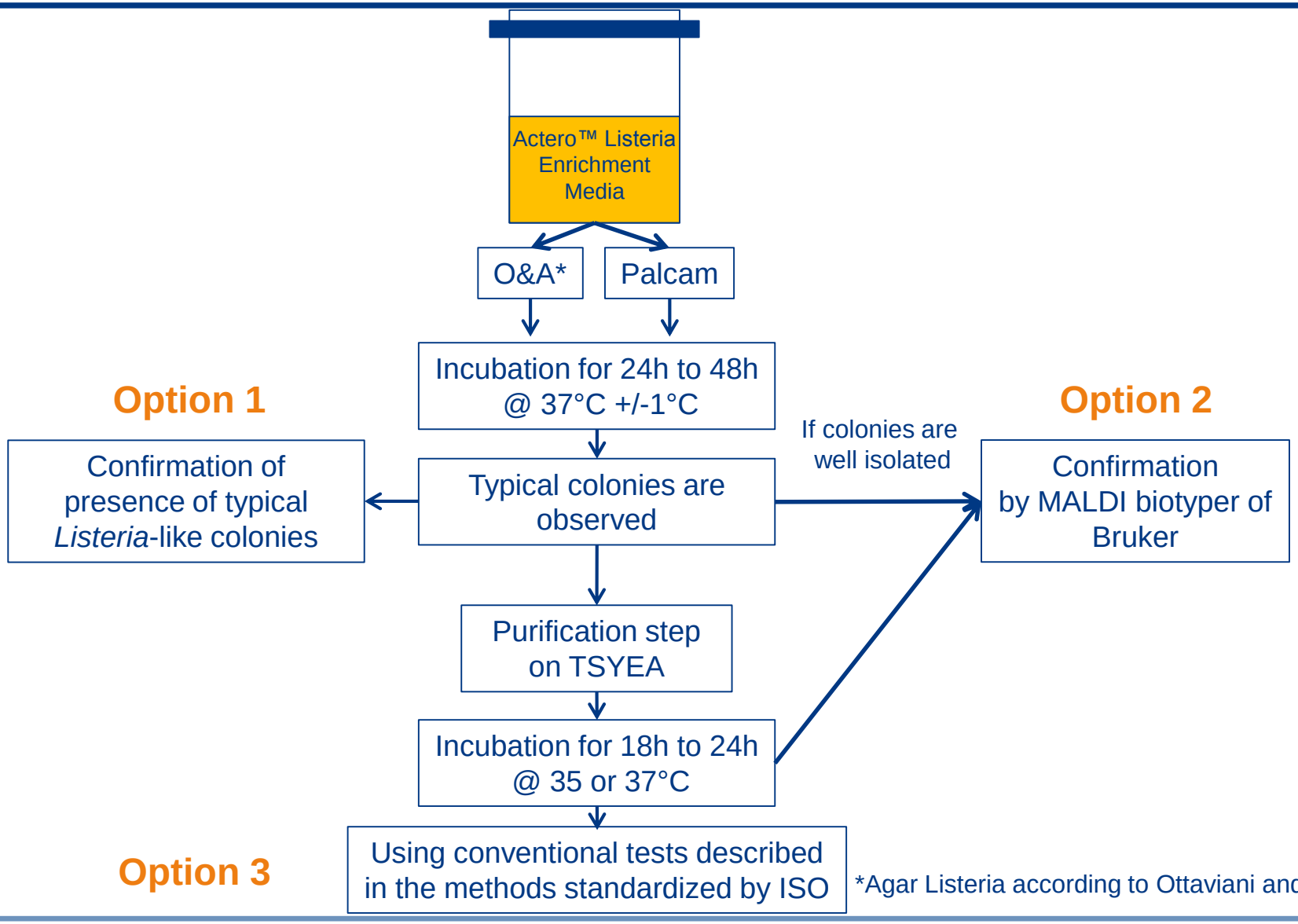
Defects of these methods

- Long time of answer
- High cost/price
- High technicality
- Difficulty of interpretation
- Sometimes unreliable

And regularly the result is not clear or unsatisfactory or arrive to late…

Need for a more reliable, faster and cheaper method

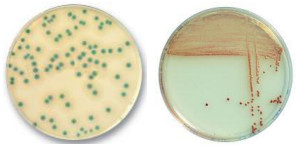
Options for Confirmation of Presumptive Positives



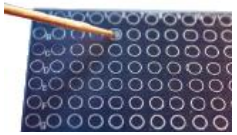
*Agar Listeria according to Ottaviani and Agosti

MALDI-TOF MS
Workflow of the method

❶ Taking pure and fresh colonies



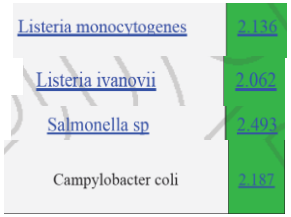
❷ Deposit and covering sample with an adapted chemical matrix



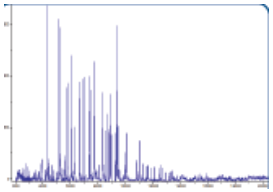
❸ Sample Analysis:

Proteins are desorbed, charged with one or two protons and accelerated under a high vacuum (5.10^{-6} mBar) in an electric field up to a detector which measures their time of flight and thus their mass. All the analyzed proteins form a characteristic spectrum of each germ (of 2KDa to 20 KDa)

❺ Automatic Identification



❹ Processing of the spectra and comparison to the MALDI-TOF Data Base: more than 7000 reference spectra covering 2400 species and 500 genus of bacteria and yeast. Continuously updated by Eurofins LMO R&D Team



MALDI-TOF MS:
Gains for Food Microbiology

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Time-to-result gains:

***Listeria* (AFNOR certified)**

- **ISO 11290-1** **14-50%**
- **BACGene *Listeria* PCR** **~ 25%**

Salmonella

- **ISO6579** **20-50%**
- **BACGene *Salmonella* PCR** **~ 25%**

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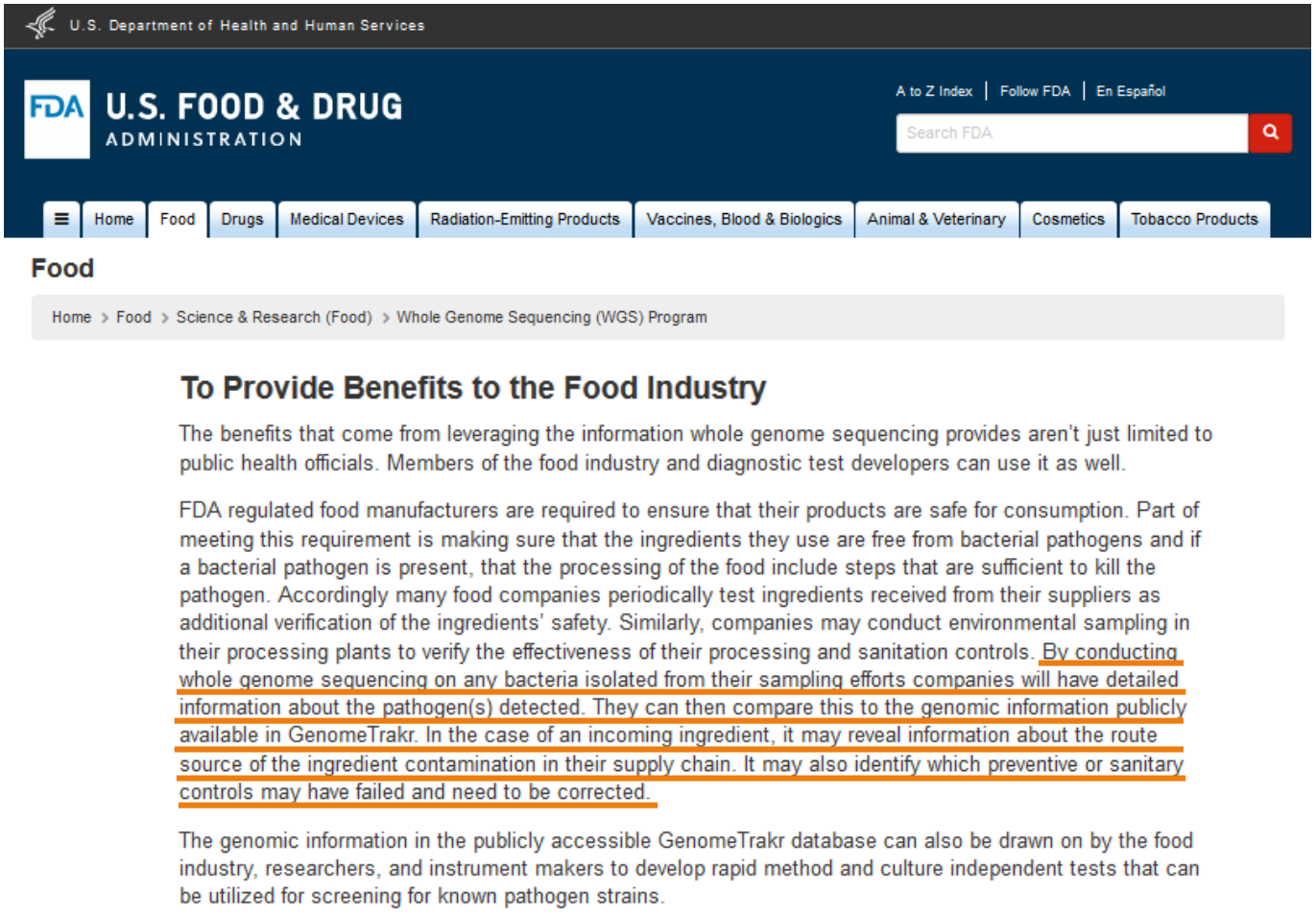
What Benefits?

Whole Genome Sequencing is used and recommended by the FDA:



U.S. Department of Health and Human Services

FDA U.S. FOOD & DRUG ADMINISTRATION

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Food

Home > Food > Science & Research (Food) > Whole Genome Sequencing (WGS) Program

To Provide Benefits to the Food Industry

The benefits that come from leveraging the information whole genome sequencing provides aren't just limited to public health officials. Members of the food industry and diagnostic test developers can use it as well.

FDA regulated food manufacturers are required to ensure that their products are safe for consumption. Part of meeting this requirement is making sure that the ingredients they use are free from bacterial pathogens and if a bacterial pathogen is present, that the processing of the food include steps that are sufficient to kill the pathogen. Accordingly many food companies periodically test ingredients received from their suppliers as additional verification of the ingredients' safety. Similarly, companies may conduct environmental sampling in their processing plants to verify the effectiveness of their processing and sanitation controls. By conducting whole genome sequencing on any bacteria isolated from their sampling efforts companies will have detailed information about the pathogen(s) detected. They can then compare this to the genomic information publicly available in GenomeTrakr. In the case of an incoming ingredient, it may reveal information about the route source of the ingredient contamination in their supply chain. It may also identify which preventive or sanitary controls may have failed and need to be corrected.

The genomic information in the publicly accessible GenomeTrakr database can also be drawn on by the food industry, researchers, and instrument makers to develop rapid method and culture independent tests that can be utilized for screening for known pathogen strains.

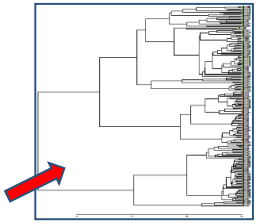
Long-Term Surveillance by WGS

How does is work?

- You have a positive pathogen finding (e.g. Listeria)

- The isolate is picked up and sent to us

- The genome of the isolate is fully sequenced (Whole Genome Sequencing = WGS)



- Sequence Comparison with
  - your Private Pathogen Library and/or
  - GenomeTrakr Entries from the FDA

2015 2016

- Output: Same Strain? yes/no

Summary

Rapid Detection

- Fast enrichment of *Listeria*
- Innovative 2-1 multiplex PCR
  - *Listeria spp.*
  - *Listeria monocytogenes*

Rapid Confirmation

- MALDI-TOF reduces confirmation time

Long-Term Surveillance

- Whole Genome Sequencing

Eurofins Worldwide

Laboratories involved in this presentation:

Eurofins GeneScan
Freiburg, Germany

Eurofins Laboratoires de Microbiologie Ouest
Nantes, France

Eurofins Genomics
Ebersberg, Germany

Further Questions - Contacts

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BAC*Gene Listeria* Multiplex, MALDI-TOF

Thank you for your attention!

Questions?