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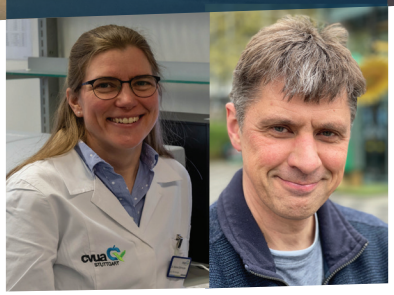

Expert Insights

Differentiation of *Listeria monocytogenes* strains by their serogroup using infrared spectroscopy

Report from our everyday laboratory life

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GP

The bacterial pathogen *Listeria monocytogenes* (*L. monocytogenes*) is often transmitted through food. Individual *Listeria* strains repeatedly cause outbreaks of illness, potentially affecting numerous people, and in rare cases even resulting in death. To investigate such outbreaks and take countermeasures, it is important to be able to distinguish between different bacterial strains of the same species.

For *Listeria*, Fourier transform infrared (FTIR) spectroscopy can be used for rapid serogroup typing. In our food safety/microbiology laboratory, we conducted thorough testing of a spectrometer software system that has been trained using artificial intelligence to assess its performance. The result: everything correct. The procedure is an integral part of our accredited food analysis workflow.

The CVUA Stuttgart is the central laboratory for food disease samples in Baden-Württemberg, Germany: All foods that are suspected of being causally involved in a human disease case in the southwest of the republic are examined here.
In our food microbiology laboratory, we analyze around 1,800 food samples for *Listeria* every year. This includes both disease-related food samples and risk-based planned samples. The examination procedure used is specified for us by the international standard ISO 11290 [1, 2]. If the selective agar plates during the workflow show presumptive *Listeria* colonies, these must be confirmed in further steps. To do this, the isolates first undergo a mass spectrometric MALDI-TOF analysis in our identifying spectroscopy laboratory, in which the bacterial species is determined. If the suspicion of *L. monocytogenes* is confirmed, we include FTIR spectroscopy, a fast, simple, and cost-effective method for fine differentiation even below the species level (for different serogroups, pathogenicity factors, vaccine strains) [3–5].

So far, four different serogroups (SG) have been described for *L. monocytogenes*: the combination group 1/2, as well as groups 3, 4 and 7. The two serogroups 1/2 and 4 are most frequently involved in human disease outbreaks and are therefore particularly relevant.
To distinguish between different *L. monocytogenes* strains based on their serogroups, a so-called classifier has now been tested in our laboratory, which is commercially available from the manufacturer of the infrared spectrometer, Bruker Daltonics, Bremen. The classifier essentially consists of an artificial neural network that was trained until it achieved the best possible discriminatory power between the different serogroups. For us, the complete solution consisting of an FTIR device and classifier had to pass the practical test in a formal validation, which makes it easier to use in our accredited laboratory environment.

The selectivity of FTIR spectroscopy in distinguishing between different serogroups was checked in an external validation based on the guidelines for MALDI-TOF validation [6]. For this purpose, 94 different *L. monocytogenes* strains from our in-house culture collection were used. The serogroup of these had previously been determined by the National Reference Laboratory for *L. monocytogenes* at the Federal Institute for Risk Assessment in Berlin. All serogroups were represented in these strains except SG 7, which occurs extremely rarely and has never been detected in a sample in our laboratory.

A total of 630 infrared spectra were created from these strains and typed by the classifier. The true positive rate of assignment was 100% for serogroups 1/2 and 4. During the validation process, the rare serogroup 3 and the even rarer serogroup 7 were combined, which resulted in a true positive rate for the SG 3&7 combined group of 100%. The separation of the two serogroups 3 and 7 with this classifier has not yet been shown - due to a lack of isolates. However, this is not relevant in practice due to their rarity.

With the now successfully validated classifier, *L. monocytogenes* isolates can be quickly and cost-effectively differentiated in our laboratories based on their serogroup affiliation. If necessary, as a next step we may subject the infrared spectra to a spectroscopic cluster analysis and thus very easily and reliably select the isolates that are representative of the sample. These are then classified even more precisely using whole genome sequencing (WGS). Through this overall workflow, we can make an important contribution to clarifying listeriosis disease outbreaks or contamination routes in food processing plants.

The above described work was recently published in the journal of Clinical Spectroscopy [7].

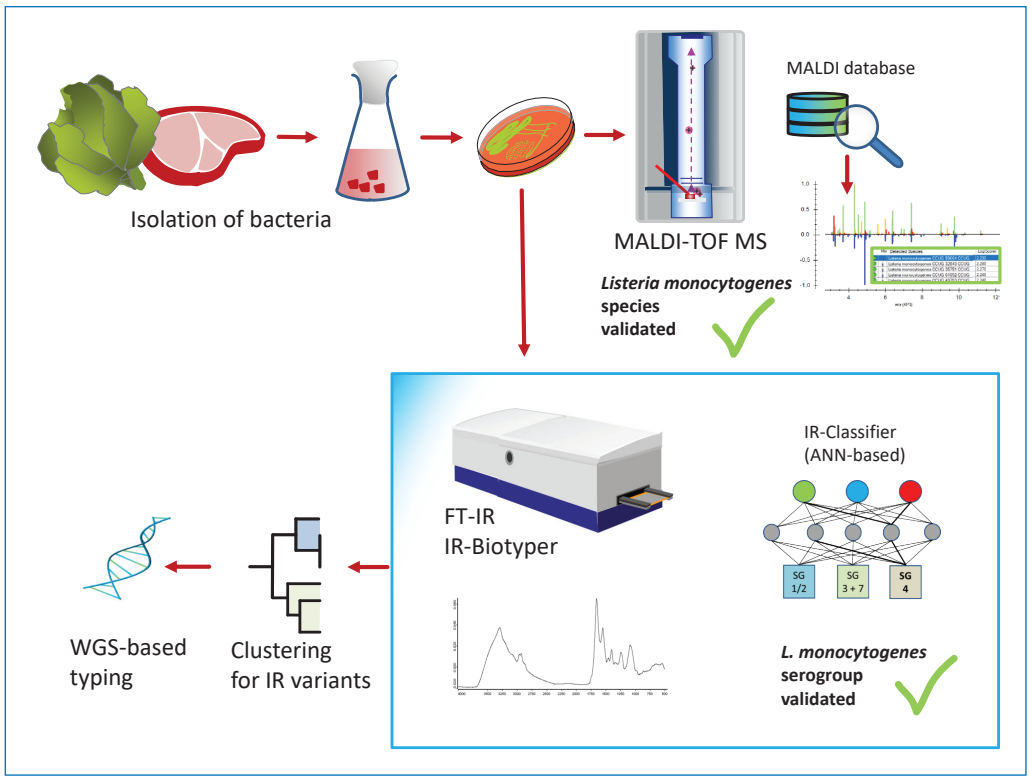

Figure 1:
From food to screened pathogen: workflow for the isolation and fine typing of *Listeria monocytogenes* in the laboratories for food microbiology and identifying spectroscopy at the CVUA Stuttgart (Copyright of figure by CVUA Stuttgart).

Infobox

Listeria monocytogenes

... is a well-researched pathogen for which around 2,000 cases are reported in Europe each year. *Listeria* is widespread in the environment and quite robust to living conditions usually difficult for microbes, such as freezing, drying and treatments with acid or alcohol. Particularly when protected in biofilms, they can be unpleasantly resistant to cleaning and disinfecting agents. In addition, they can reproduce, albeit slowly, even at refrigeration temperatures. *Listeria* is generally transmitted through contaminated food. Healthy adults may only experience mild flu-like symptoms when infected with *L. monocytogenes*. However, immunocompromised people (small children, old and chronically ill people as well as pregnant women) may experience significantly more serious health consequences from listeriosis, such as gastrointestinal symptoms, sepsis, and meningitis. In pregnant women, an infection may even result in a fetus's abortion. To protect against foodborne infections, it is recommended to avoid consuming selected foods [8].

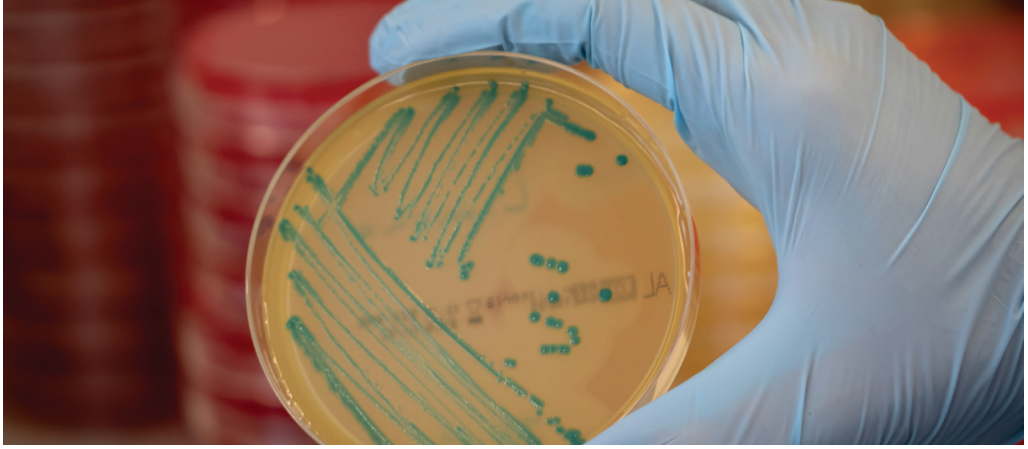

Figure 2:
L. monocytogenes on ALOA Agar (Copyright of figure by CVUA Stuttgart)

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