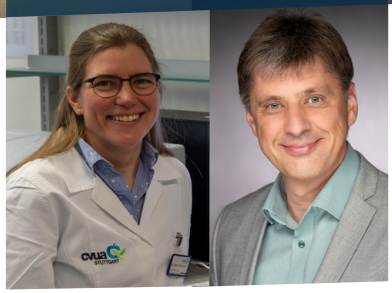
Dr. Helene Oberreuter and Dr. Jörg Rau



Expert Insights

Validation of a new infrared spectroscopy method for distinguishing important Salmonella serogroups

Report from our everyday laboratory life

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GP

Salmonella species are among the most prominent foodborne pathogens worldwide. Ensuring reliable identification and precise serogrouping is therefore essential to protect consumers from contaminated products. Conventional serotyping, however, depends on specialized antisera and involves considerable manual effort.

Fourier-transform infrared (FTIR) spectroscopy now offers laboratories a practical, easy-to-implement alternative for key *Salmonella* serogroups. At the Chemical and Veterinary Analysis Agency (CVUA) Stuttgart, we have validated a new AI-based *Salmonella* classifier for the IR Biotyper®. In evaluating the four most common serogroups, the system proved suitable for routine use and can now be integrated into daily analyses - helping laboratories reduce hands-on time and overall costs.

The CVUA Stuttgart is the central laboratory for food disease samples in Baden-Württemberg, Germany: Food items from anywhere within the state of Baden-Württemberg that are suspected of having caused illness in humans are analyzed there.
Salmonella are among the primary targets of investigation. It is therefore especially important to use up-to-date methods for this purpose. Fourier-transform infrared spectroscopy (FTIR) uses infrared radiation (thermal radiation) to capture the overall biochemical composition of microorganisms. Standardized growth conditions ensure reproducible IR spectra that show patterns suitable for sub-species-level typing. *Salmonella* serogroups also exhibit these characteristic "fingerprints."

Using modern software supported by artificial neural networks, identification methods can be established on this basis. Independently and autonomously from the instrument manufacturer, we demonstrated in an extensive evaluation that the method performs its task to a very high degree of reliability. We aligned the procedure with the high standards of the German national guideline for validation of MALDI-TOF MS methods.[1]
Formal validation is a prerequisite for implementation in our accredited routine laboratory. We have summarized the results of this study in CVUA Stuttgart's e-Journal No. 16.[2] The now comprehensively validated classification method can be applied for rapid and simple differentiation of the most common *Salmonella* serogroups, for example in our food control or animal health diagnostic laboratories.

The work presented here used the IR Biotyper, an infrared spectrometer from Bruker, as the instrument platform. For the method, initially a classifier capable of distinguishing 36 different *Salmonella* serogroups had been developed by Bruker. An FTIR classifier is a software tool based on a classifying artificial intelligence (AI) that separates predefined classes based on their infrared spectra. In a second step, we assessed the performance of this classifier for differentiation by means of an extensive external single-laboratory validation. This validation was carried out according to the guidelines for validating species identification by MALDI-TOF MS in single laboratories or laboratory networks, published by the German Federal Office of Consumer Protection and Food Safety, and adapted for targeted identification.[1] As target parameters, the *Salmonella* serogroups most frequently detected in the EU - O:4 (B), O:6,7 (C1), O:8 (C2–C3), and O:9 (D1) - were selected and validated.

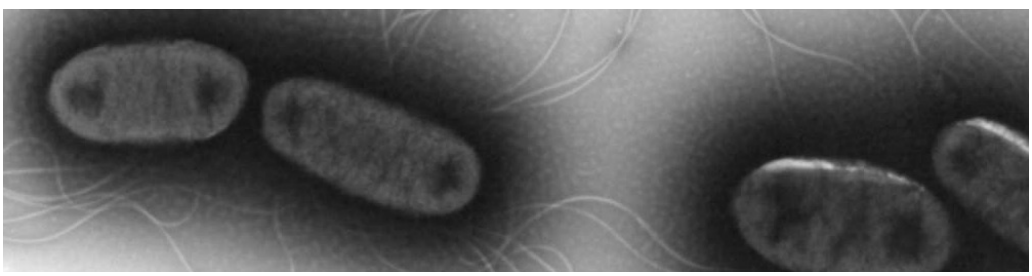

Figure 1: *Salmonella* Enteritidis visualized by electron microscopy, 20,000× magnification. (Copyright of figure by CVUA Stuttgart)

Infobox

Salmonella

... are widespread in nature and among the most important bacterial agents causing diarrheal disease in humans. Approximately 2,600 different serovars are currently described, determined via agglutination with rabbit antisera. Salmonella are usually transmitted through animal-derived foods, but also through plant-based foods, and cause diarrhea - often accompanied by fever - typically one to three days after ingestion. Their spread is frequently favored by inadequate refrigeration of food; hence, particularly in summer, rapid multiplication of the organisms quickly leads to a critical infectious dose.[3,4]

For this investigation we used 1,039 infrared absorption spectra from 167 *Salmonella* strains belonging to 39 serogroups. The results for serogroups O:4, O:6,7, and O:9 met or exceeded the adapted guideline requirements, each achieving an inclusivity of >99%. Because of a single divergent strain, the validation results for serogroup O:8 were only slightly weaker, with a true-positive rate of 96.1% (Table 1). The fully validated classification method can now be used in routine analysis for rapid and simple differentiation of the most common *Salmonella* serogroups - for example, in our food surveillance or animal health diagnostic laboratories. The generated IR spectra can subsequently be pre-selected via cluster analysis after serogroup assignment, allowing targeted downstream fine-typing, such as whole-genome sequencing. This substantially reduces the workload in routine analysis (Figure 2).

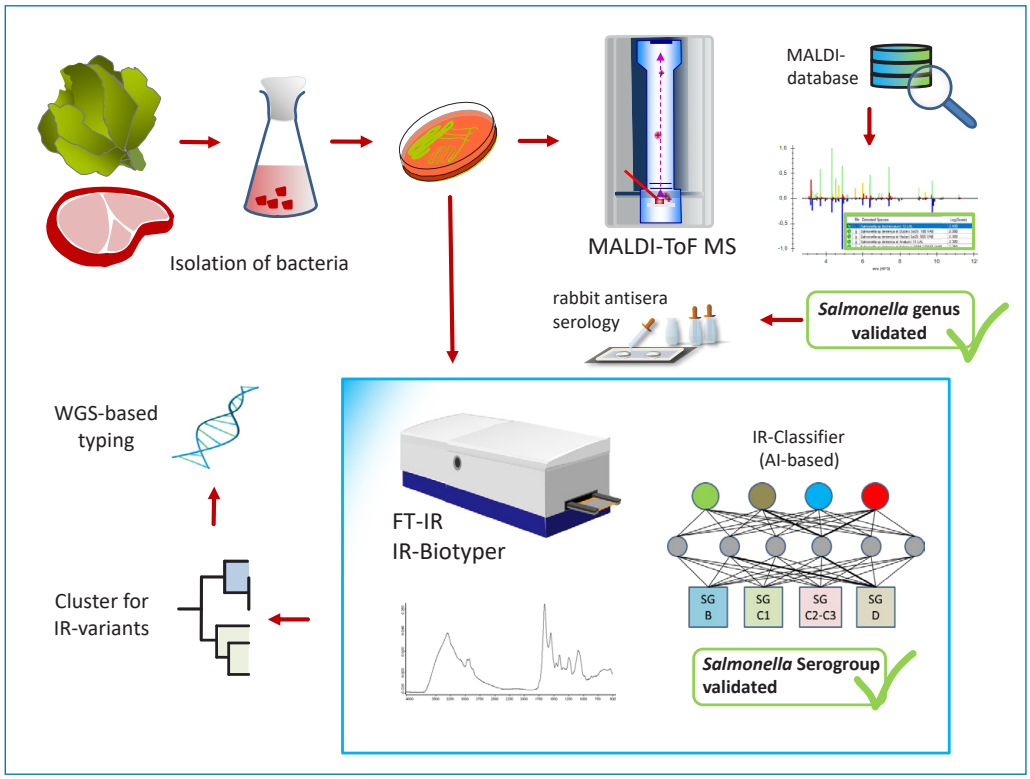

Figure 2:
Workflow for the isolation and fine typing of *Salmonella* species from food and animal samples at the CVUA Stuttgart. (Copyright of figure by CVUA Stuttgart)

Table 1: Results of *Salmonella* serogroup identification. True: The respective individual spectrum was correctly assigned to its serogroup. False: The respective individual spectrum was assigned to another serogroup. "Positive" refers to the target parameter; "negative" to the non-target parameter (control group).

Extensive supplementary materials, e.g. calculation sheets, are accessible for download at https://maldi-up.ua-bw.de/

Target parameter	SG O:4 (B)	SG O:7 (C1)	SG O:8 (C2-C3)	SG O:9 (D)
No. of isolates of the target parameter	25	25	25	25
No. of isolates of the target parameter	162	150	159	159
No. of identified spectra	162	148	154	159
Identification rate (%)	100	98.7	96.9	100
True positives	162	147	148	159
False negatives	0	1	6	0
True positive rate (%) of identified spectra	100	99.3	96.1	100
False negative rate (%) of identified spectra	0	0.7	3.9	0
No. of isolates of the non-target parameter group	142	142	142	142
No. of spectra of the non-target parameter group	877	889	880	880
No. of identified spectra	840	854	848	843
Identification rate (%)	95.8	96.1	96.4	95.8
True negatives	839	854	848	837
False positives	1	0	0	6
True negative rate (%) of identified spectra	99.9	100	100	99.3
False positive rate (%) of identified spectra	0.1	0	0	0.7

References

[1] J. Rau, Dolch LJ, Eisenberg T, et al., Guidelines for validating species identifications using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) in a single laboratory or in laboratory networks. MALDI-TOF-MS working group pursuant to § 64 of the German Food and Feed Code (LFGB), Berlin (BVL), Editor. 28 Oct 2022.

[2] Oberreuter H., Cordovana M., Dyk M., Rau J., Establishment and Thorough External Validation of an FTIR Spectroscopy Classifier for Salmonella Serogroup Differentiation. Aspects of Food Control and Animal Health, 2025. 16: p. 1–18. https://ejournal.cvuas.de/issue202516.asp.

[3] Bundesinstitut für Risikobewertung BfR. Gesundheitliche Bewertung von Salmonellen.

[4] Grimont P.A.D. & Weill F.-X. Antigenic Formulae of the Salmonella Serovars, 2007.

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