news digest #014

Same-day *Salmonella* confirmation and strain discrimination, starting from colony material – isn't that egg-citing?

Eggs are a widely used food item globally and are consumed in a variety of ways. They are a good source of proteins, vitamins, and minerals and are used in cooking and baking to add flavor, texture, and nutrition. In many cultures, eggs are a staple breakfast food and are commonly used in dishes such as omelets, frittatas, and scrambled eggs. "Tamago kake gohan" is a popular Japanese breakfast consisting of cooked rice topped or mixed with raw egg and soy sauce. Eggs are also used as an ingredient in a wide range of baked goods, including cakes, cookies, and pastries. Hungry yet?

Despite this egg-straordinary hymn on eggs, they may unfortunately also bring trouble.

Eggs have the potential to carry harmful bacteria such as *Salmonella*, which can cause foodborne illness. To prevent such illnesses, it is important to handle, prepare, and store eggs properly. For instance, refrigerate eggs, cook them thoroughly, and dispose of any cracked or dirty eggs. *Salmonella* is not limited to egg-based products; it can also be present in animal farms and poultry meat. The illness is commonly contracted by consuming contaminated foods, including eggs, poultry, beef, pork, cocoa, sweets, vegetables, and sprouts. Recently, there have been outbreaks related to chocolate products, such as the 2022 multi-country *Salmonella* outbreak linked to chocolate products reported by the EFSA.[1] In the US, there was a multi-year, multi-state outbreak of *Salmonella* infections traced to raw turkey products.[2]

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When conducting *Salmonella* testing in the laboratory, a sample is taken from either the specimen (such as chicken skin, chicken meat, egg or cocoa) or the environment (including water, dust samples, feces samples, or production areas in the food industry). The sample is then either cultured or subjected to a highly sensitive molecular test. Based on the test results, further confirmation or additional testing may be required to determine the specific strain of bacteria present.

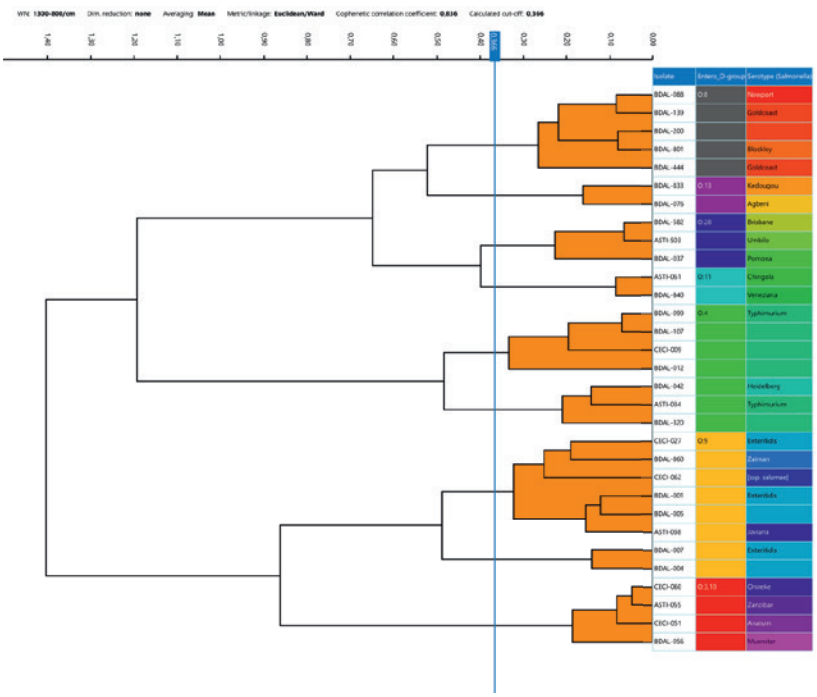
In the European Union, food samples are subject to regulation No. 2073/2005, which sets legal microbiological criteria for determining the acceptability of food. Each foodborne microorganism has its own analytical reference, such as ISO 6579 for *Salmonella*.

To validate confirmation methods that can advance a suspected result to a confirmed one, the International Organization for Standardization (ISO) has developed a dedicated standard; ISO 16140-6. Bruker's MALDI Biotyper® has been validated and certified by MicroVal, for confirmation of *Salmonella* spp., in accordance with ISO 16140-6. The MALDI Biotyper solution is an innovative mass spectrometry-based solution that can identify microorganisms, starting from microbial cultures. With a workflow that only requires a few steps, it can generate a high-quality and reliable microorganism identification from non-selective cultures or confirmation within minutes after detecting a positive selective culture. As a result, this technology is ideal for food laboratories seeking to avoid time-consuming confirmation methods on species level.

To identify the specific serotype of *Salmonella* present, a more in-depth characterization is necessary. One method for subtyping is serotyping, which is based on differences in bacterial surface antigen composition. In this case, serology refers to the antibodies formed as a result of a *Salmonella* infection. During a serotyping test, *Salmonella* isolates are analyzed for reactivity with specific anti-*Salmonella* antibody-containing sera. Public health scientists have used serotyping tests since the 1960s to help identify *Salmonella* outbreaks and track them to their sources. The Atlas of *Salmonella* in the United States, 1968-2011, summarizes 42 years of laboratory-confirmed surveillance data on *Salmonella* isolates from humans, including analyses by age, sex, geography, and season of the year.[3] In the EU, an increase in confirmed human salmonellosis cases after 2014 prompted an investigation into contributory factors and control options in poultry production. The three main target serovars for breeding hens are *Salmonella* Enteritidis, *Salmonella* Typhimurium (including monophasic variants), and *Salmonella* Infantis.[4]

When a specific serotype of *Salmonella* increases, it raises concerns of an outbreak and prompts investigation by "disease detectives". To detect outbreaks as quickly as possible, laboratories use various technologies. One such technology is Fourier transform-infrared (FT-IR) spectroscopy, applied by the Bruker IR Biotyper®, which types *Salmonella* O-serotypes from the same colony material used for confirmation. Unlike the MALDI Biotyper, the IR Biotyper does not provide a reference library for identification of different organisms. However, it enables cluster analysis of different isolates, revealing if they originate from the same or different sources, thus indicating a potential outbreak by source tracking.

Additionally, the IR Biotyper software includes an Artificial Neural Network (ANN) classifier that discriminates O-serotypes. Different serovars are part of one O-serogroup, e.g., *S.* Enteritidis is a member of the O:9 group. The IR Biotyper supports clustering of local isolates for smarter discrimination of O-serogroups and local serovars, allowing for rapid discrimination prior to genomic technologies such as NGS or WGS are applied to unknown *Salmonella* samples.

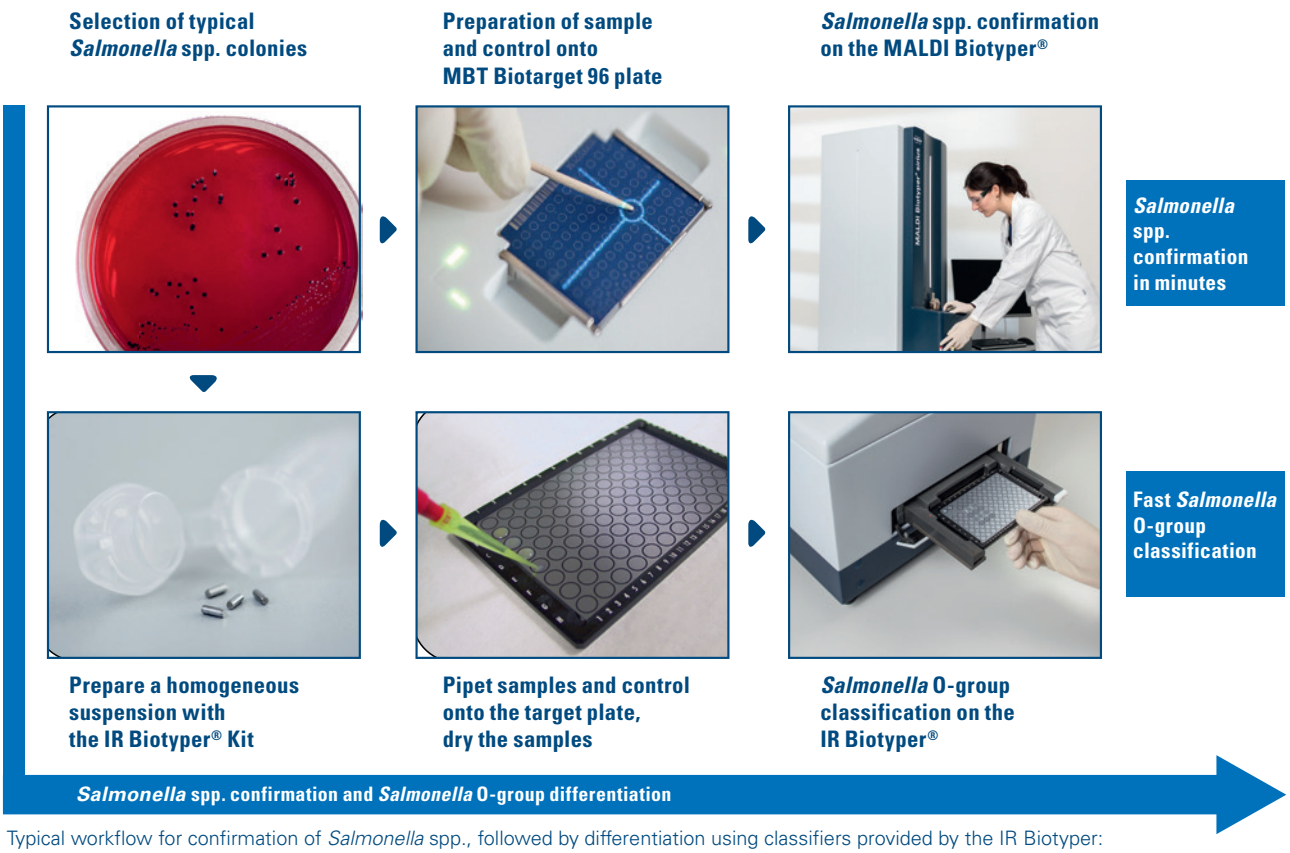


Hierarchical cluster analysis of average spectra (3 technical replica) from 31 serotyped *Salmonella enterica* isolates grown on McConkey agar. IR clustering is mostly influenced by the isolate's O-group (somatic antigen). O-serogroups are clustered and shown in different colors (grey, pink, blue, light blue, green, yellow, red).

The IR Biotyper offers a user-friendly and streamlined workflow for the rapid strain typing of microbial isolates. To begin the process, a homogeneous suspension is prepared from the colony, using the IR Biotyper Kit. Aliquots of the suspension are then pipetted onto a sample plate along with a control, and after drying, the sample plate is inserted into the IR Biotyper for analysis. The entire sample preparation process takes only 30 minutes and can handle up to 30 microbial isolates.

With the latest software version, the newly integrated classifier allows *Salmonella* isolates to be classified as an immediate match to *Salmonella* reference spectra, using an Artificial Neural Network (ANN) classification model. This model can be continually improved and trained with common findings in veterinary and food applications, resulting in improved classification power not only for *Salmonella*, but for other organisms as well.

The combination of the MALDI Biotyper (using MALDI-TOF mass spectrometry) for rapid microorganism identification and the strain typing/differentiation capability of the IR Biotyper (using Fourier Transform - Infra-Red (FT-IR) spectroscopy) provides a seamless, fast, and easy workflow solution for microbial identification/confirmation and subsequent strain typing. The FT-IR technology is particularly efficient for studying the epidemiology of *Salmonella* outbreaks in a rapid pre-screening.



Typical workflow for confirmation of *Salmonella* spp., followed by differentiation using classifiers provided by the IR Biotyper: Starting from colony material and using both instruments in parallel or sequential, *Salmonella* spp. confirmation and differentiation of O-serogroups can be achieved very fast, starting from colony material.

References

1 European Centre for Disease Prevention and Control, European Food Safety Authority, 2022. Multi-country outbreak of monophasic *Salmonella* Typhimurium sequence type 34 infections linked to chocolate products, first update - 18 May 2022.

2 Hassan R et al., Multistate Outbreak of *Salmonella* Infections Linked to Raw Turkey Products — United States, 2017–2019. MMWR Morb Mortal Wkly Rep 2019;68:1045–1049. http://dx.doi.org/10.15585/mmwr.mm6846a1

3 https://www.cdc.gov/salmonella/pdf/salmonella-atlas-508c.pdf

4 *Salmonella* control in poultry flocks and its public health impact. EFSA Panel on Biological Hazards (EFSA BIOHAZ Panel), 2019, https://doi.org/10.2903/j.efsa.2019.5596

Not for use in clinical diagnostic procedures.
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