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Expert Insights

IR Biotyper®: differentiating between pathogenic bacteria to protect animal health and control infectious diseases

GP

Innovation with Integrity

About Annet Heuvelink Ph.D.

Dr. Heuvelink started her career in food microbiology. Having completed a Ph.D. in verocytotoxin-producing *Escherichia coli* (*E. coli*) in food production at Radboud University Medical Center, Nijmegen, the Netherlands, Dr. Heuvelink worked for the Dutch National Institute for Public Health and the Environment (RIVM) and later studied zoonotic bacterial pathogens at the Netherlands Food and Consumer Product Safety Authority (NVWA). In 2011, Dr. Heuvelink switched from human health to animal health, joining Royal GD as Senior Scientist, Bacteriology. Dr. Heuvelink explains her role at Royal GD:

"I work in research and development (R&D) and am responsible for innovation in the laboratory: my job is to ensure that the animal health service is operating to the highest quality standards and continuously improving using breakthrough microbiological tools and technology. Our team's expertise in veterinary bacteriology supports both internal and external projects throughout the organization."

About Royal GD

Backed by over a century of experience from its origins in 1919, when Dutch farmers and dairy organizations joined forces to combat animal diseases, Royal GD is a world leader in animal health. With state-of-the-art veterinary laboratories, R&D facilities and veterinary knowledge and expertise, its services support governments, veterinarians and farmers worldwide with animal health programs, consultancy and laboratory diagnostic services. Dr. Heuvelink highlights the importance of her work:

"Animal health management protects the animals themselves and contributes to sustainable farming. It ensures we meet the goals of a society that demands safe food that is produced in a responsible, environmentally-friendly way."

One of the largest veterinary laboratories in the world lies at the heart of the Dutch animal health service, Royal GD. Serving animal owners, veterinary practices, and governments, around 400 employees work daily on the health of livestock and companion animals, conducting 4.5 million tests per year.

Introduced at Royal GD in 2011, the Bruker MALDI Biotyper® was first deployed in addition to traditional biochemical identification methods to provide faster and more uniform results for routine microbiology diagnostics, and it fast became the laboratory's preferred testing method. In 2023, as a response to increased demand for management of outbreaks of infectious diseases in animals, the laboratory then acquired a microbial strain typing solution, the Bruker IR Biotyper®, with the goal of enhancing strain typing capabilities and pathogen differentiation.

Routine testing and innovation

Royal GD fosters collaboration through a cross-departmental structure, comprising diagnostics, R&D and animal health experts.

Dr. Heuvelink explains: *"Communicating with the veterinarians in the field allows us to understand market needs first-hand from those on the ground. The R&D department can then tailor its research accordingly to track and anticipate market needs through pushing the boundaries of veterinary science."*

The MALDI Biotyper harnesses matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) technology to bring new speed and accuracy to routine microorganism identification. As an experienced user, Dr. Heuvelink has seen the advantages MALDI-TOF technology offers over traditional biochemical identification techniques and molecular tests such as polymerase chain reaction (PCR).

She says: *"The MALDI Biotyper is significantly faster than identification by biochemical or PCR testing, involves a uniform procedure for all bacterial species and provides accurate results, making it the gold standard for veterinary bacterial identification. The simple workflow is fully traceable, while the extensive reference libraries offer comprehensive coverage of more than 4,700 microorganisms. We are really satisfied with its integration and performance, and even the most traditional of our microbiologists now use it as their preferred method."*

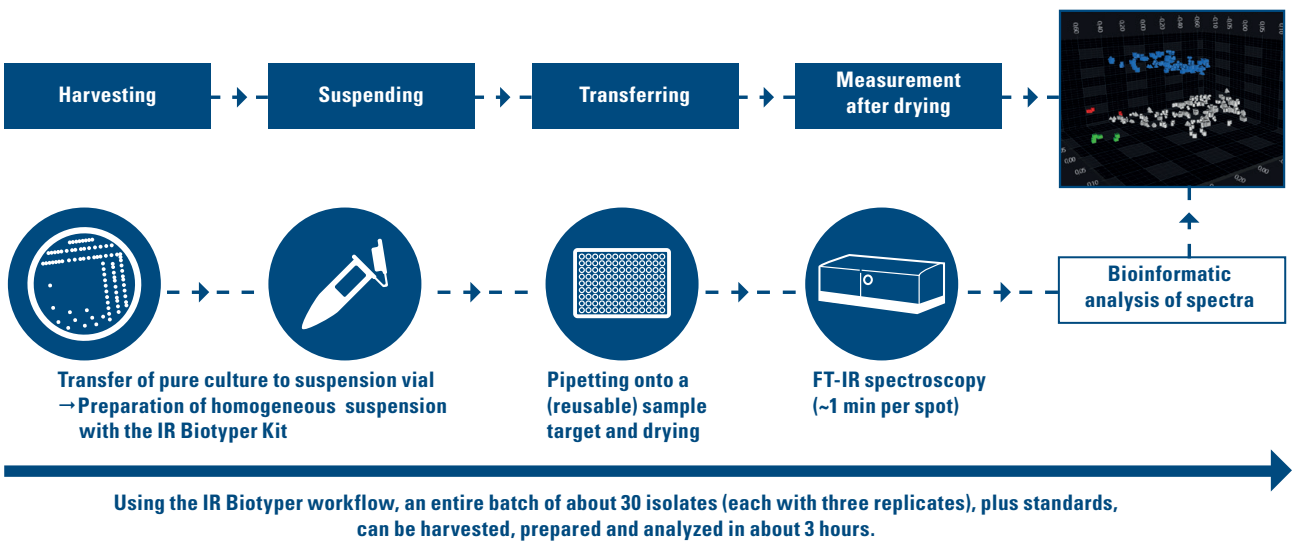
Following the success of the MALDI Biotyper, Dr. Heuvelink was keen to explore how the IR Biotyper, based on Fourier-transform infrared (FT-IR) spectroscopy, could address field needs for quick and accurate strain differentiation.

The benefits of FT-IR in microbiology

FT-IR spectroscopy is a technique that makes it possible to classify infrared spectra of different bacteria into clusters, according to their similarities. Scientists at Royal GD use the results to create customer-specific bacterial typing approaches for the isolated bacterial strains.

Simply identifying the presence of a bacterium does not always provide sufficient information to implement an effective containment and treatment strategy. Instead, it is increasingly necessary to differentiate between specific bacterial strains, for example to establish an epidemiological link, or differentiate strains based on their serotype, virulence properties or resistance to certain therapeutic agents. FT-IR spectra of varying strains belonging to the same bacterial species often exhibit sufficient differentiation to allow a distinction to be made between these strains. The Royal GD scientists use cluster analysis techniques to analyze the generated spectra and determine whether animal infections are caused by related strains or different strains each time.

Figure 1
FT-IR workflow for bacterial strain differentiation.



Introducing the technology to veterinary diagnostics

Bruker invited Dr. Heuvelink and her technician to test isolates using an IR Biotyper at its Bremen facility. Encouraged by the results, Royal GD trialed the instrument in its R&D laboratory and conducted extensive validation for three months prior to full implementation in 2023. Dr. Heuvelink explains: *"We were an early adopter of the IR Biotyper for veterinary diagnostics, at a time when bacterial typing diagnostics were almost unheard of in veterinary microbiology. Our testing and adoption of FT-IR technology has allowed us to respond to questions in the field more efficiently and expand into new applications, meeting emerging market demand across our customer base."*

Provided a standardized approach is used, results obtained using FT-IR spectra are highly reproducible and can help trace the source of a farm outbreak, for example. Dr. Heuvelink explains: *"The IR Biotyper can be used to find possible relationships among bacterial isolates faster and at a significantly lower cost than conventional methods such as DNA sequencing analyses."*

Using the IR Biotyper, Royal GD offers cluster analysis for an extensive range of bacterial species, including *E. coli* and *Klebsiella pneumoniae* (*K. pneumoniae*), and has developed a serotyping method for *Streptococcus suis* (*S. suis*). Dr. Heuvelink adds: *"Strain typing with the IR Biotyper allows us to accurately differentiate between various strains and we have been impressed by its ease-of-use, versatility and speed."*

Case study: Bovine *K. pneumoniae* mastitis

Recently, the IR Biotyper played a role in a bovine *K. pneumoniae* mastitis outbreak investigation at Royal GD. Mastitis caused by the ubiquitous *K. pneumoniae* is a major challenge for many dairy farmers and can prove fatal in some cases.[1] Vaccination does not guarantee protection and treatment results for *K. pneumoniae* udder infections with antibiotics are often disappointing. Surviving cows often develop chronic mastitis. Because mastitis not only threatens the health of the herd but also adversely affects both milk yield and quality, efficient outbreak detection is vital from both an economic and an animal health perspective.[2]

A farm affected by an outbreak of *K. pneumoniae* contacted Royal GD for urgent support. Dr. Heuvelink explains the project: *"We wanted to compare the* K. pneumoniae *isolates from infected cows to determine whether the milking apparatus was the source (identical isolates; cow-to-cow transmission) or whether environmental conditions were to blame (different isolates; different sources). Previously, we would have used either random amplified polymorphic DNA-PCR (RAPD-PCR) or pulsed-field gel electrophoresis (PFGE) for this type of investigation, but both have significant limitations; PFGE typically takes up to a week, while RAPD-PCR testing requires species-specific primers and so is costly in terms of consumable reagents."*

Providing a new alternative, the IR Biotyper allowed Royal GD to quickly and accurately compare *K. pneumoniae* isolates from samples collected at the farm (for example milk, bedding material and feces). Dr. Heuvelink describes the outcome of the investigation: *"The IR Biotyper reliably confirmed the presence of multiple* K. pneumoniae *strains circulating in the farm and causing mastitis and, since the implementation of a treatment and hygiene management plan, no recurring cases have been identified."*

Following the set-up and validation of a uniform method with species-specific incubation requirements, the IR Biotyper delivers reproducible results across a range of bacterial species found in animal infections. This means typing analyses can be performed with minimal set-up time, yielding valuable insights while making considerable time and cost savings.

Case study: Poultry *E. coli*

The IR Biotyper is instrumental in a collaborative research project exploring *E. coli* in poultry, undertaken by Royal GD, the Faculty of Veterinary Medicine at Utrecht University and a large poultry veterinary practice. While many *E. coli* strains are not pathogenic, some can cause disease leading to reduced egg production and increased mortality.[3] To improve disease management, this study aimed to ascertain whether *E. coli* infections in the poultry meat production chain are transmitted vertically, from parent stock flocks to their offspring, or horizontally, introduced by the environment.

As part of the project, Dr. Heuvelink and her team were required to analyze around 3,500 *E. coli* isolates. She explains: *"With such a large caseload, efficient testing was essential. Although whole genome sequencing (WGS) provides reliable results, it's time- and cost-intensive. We analyzed the first 200 isolates using both WGS and the IR Biotyper. As the results were consistent, the IR Biotyper was used for the remaining isolates. We then only had to perform WGS on one or two of the isolates from each identified cluster, giving significant cost and time savings. We look forward to sharing the results of this study on the origin of* E. coli *infections in the poultry meat production chain once finalized."*

Vaccine development

Royal GD uses the IR Biotyper to improve the composition of autologous vaccines which can be used when there is no commercial vaccine available. Dr. Heuvelink explains:

"The IR Biotyper can be used to differentiate between isolates of bacterial species, such as E. coli. *It allows us to determine with confidence which isolates are closely related so that we can select and combine different isolates to make farm-specific autologous vaccines that confer the broadest-range immunity."*

The Bruker advantage

Fast, easy-to-apply and cost-effective typing methods are in high demand for routine infection control, outbreak management and vaccine development in the veterinary laboratory.

The IR Biotyper uses FT-IR spectroscopy to classify strains with high specificity and to compare isolates in the event of outbreaks. Results can then be visualized in the form of cluster analyses (dendrograms) or 3D scatter plots. In comparison with DNA sequencing, the IR Biotyper is highly versatile with one simple workflow that can be used in the routine laboratory to perform strain typing for a wide range of microbial species.

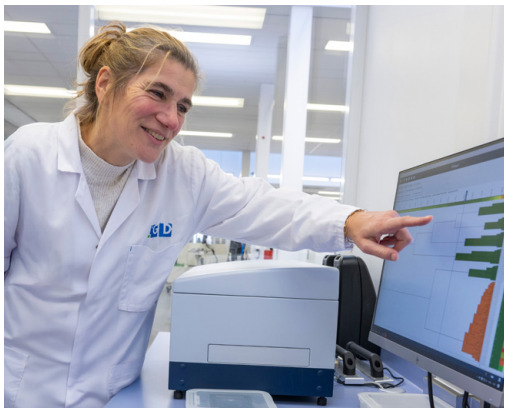
The MALDI Biotyper delivers proteomic fingerprinting using MALDI-TOF technology. Its reference libraries of more than 4,700 species (versions 2023; versions 2026 cover more than 5,300 species) are continuously maintained and updated according to strict quality-controlled procedures. The system increases the speed and accuracy of identifying microbial infection in animals, therefore preventing potential herd outbreaks or spread to humans. Data obtained using the MALDI Biotyper can be imported seamlessly into the IR Biotyper for further strain discrimination or serogrouping.

The future of IR Biotyper in animal health

Dr. Heuvelink explains her plans for the IR Biotyper: *"We have been impressed with its performance so far and look forward to using it to differentiate a broader range of bacterial species, expanding our already extensive portfolio."*

Dr. Heuvelink and her colleagues also developed an artificial intelligence (AI) model for serotyping of *S. suis* isolates. The model classifies isolates by serotype based on differences in infrared spectra found by the IR Biotyper. Dr. Heuvelink says:

"We previously performed serotyping using agglutination and PCR methods but have recently validated an AI model for serotyping of S. suis *with the IR Biotyper. We have implemented this model in the routine laboratory and plan to develop AI models for other bacterial species. We're excited to see where the IR Biotyper will take us in the future."*



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