



Expert Insights

- Microbial Confirmation and Identification in Food Microbiology Using Cutting-Edge MALDI-TOF Technology

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Bruker's MALDI Biotyper drives rapid microbial confirmation and identification in food and pharmaceuticals and receives AOAC Official Methods of Analysis at Q Laboratories.



Working with Bruker

The Microbiology Laboratory Services at Q Laboratories leads the industry in food microbiology, by providing high confidence direct confirmation and identification of microorganisms. Erin Crowley, Chief Scientific Officer of Q Laboratories, explains how the laboratory excels its identification strategy:

“By implementing Bruker’s MALDI Biotyper, we can provide our customers with fast, accurate and trustworthy molecular identification of microorganisms in their food products, allowing them to make crucial decisions rapidly.”

Microbiology Laboratory Services, Q Laboratories

Q Laboratories is an independent laboratory, providing testing and quality assurance services to the food, cosmetic, pharmaceutical, health and beauty care, and dietary supplement industries. Established in 1966, Q Laboratories has long-standing expertise in analytical testing, investing in state-of-the-art technology to best service its broad range of customers.

With several laboratories in one location in Cincinnati, Ohio, Q Laboratories is split at the top level into Microbiology and Analytical Chemistry. Within the Microbiology Laboratory Services, the Microbiology Pharmaceutical Laboratory is responsible for testing personal care products and over-the-counter pharmaceuticals, and the Microbiology Food Laboratory consists of routine food and environmental testing of samples such as flavorings and spices, baked goods, raw and ready-to-eat meats, frozen vegetables, environmental samples and water. The Microbiology

Research and Development (R&D) Laboratory conducts method validation studies, and all three feed into the Microbial Identification Laboratory, which was opened in 2017 and offers a range of customizable services, using a number of microbial techniques depending on the application.

The Microbiology Food Laboratory specializes in indicator testing, pathogen detection, environmental monitoring and allergen screening. Erin Crowley is the Chief Scientific Officer of Q Laboratories, and fellow of AOAC International, and oversees the microbiology laboratory services at Q Laboratories.

There are a total of 140 employees at Q Laboratories, about 70-75 percent of which are analysts and support personnel within the laboratories. The Microbiology Food Laboratory has approximately 17-18 analysts, and is the highest throughput laboratory on site. There is also a substantial quality assurance unit, whose primary role is to ensure the analyses are meeting regulatory standards as well as ISO 17025 accreditation requirements.

Food microbiology activity

The food microbiology work carried out at Q Laboratories is split between routine testing – where clients submit samples for confirmation and identification of microorganisms in food products – and R&D – where clients wishing to market and sell a diagnostic assay require validation needed for certification. On the routine testing side, the majority of Q Laboratories' clients are distributed throughout the United States, with approximately 60 percent within a 200 mile radius. From the perspective of R&D, the company has a global presence as an Expert Laboratory. Routine applications in the Microbial Identification Laboratory include pathogen confirmation (to confirm the presumptive result after a first screening step), and identification of spoilage contaminants (to determine the identity of unknown isolates). The routine laboratory also provides insights into trends, for example environmental contaminants in processing plants.

Introducing mass spectrometry to the laboratory

The microbial identification laboratory, which services the microbiology food laboratory, the microbiology pharmaceutical laboratory and the microbiology R&D laboratory, use a 'tool box' of methods. These include standard benchtop cultural methods, automated biochemistry, microscopic evaluation and 16S rDNA sequencing. An internal review of the technology used for microorganism identification, spurred by the

request of clients for alternative methods, led Q Laboratories to consider mass spectrometry (MS) for its identification work. Crowley explains the process behind this decision:

"We began reviewing the tools we use for microbial identification in food and pharmaceutical applications, along with the amount of isolates we were outsourcing to specialist identification labs. We wanted to improve our workflow while still providing the best results for our clients. At the same time as this internal review, we formed our Technology Advancement Group (TAG) Team who is specifically charged with looking at the technologies we currently use and those we want to potentially implement in the future.

It was a great opportunity for us to evaluate how we met client needs and the best way to modernize our laboratories.

Many clients were specifically asking for technologies by name, which was a big decision-driver."

Q Laboratories installed the Bruker matrix-assisted laser desorption/ionization (MALDI) Biotyper® (MBT) in January 2017, a microbial identification system based on MALDI-time-of-flight (TOF) MS technology, for use in its routine and R&D laboratories. The MBT determines the unique protein fingerprint of a microorganism, which is used to reliably identify a particular species by matching the test with thousands



of reference spectra from a comprehensive database. MALDI-TOF is now an important tool, and is complemented by existing techniques. For example, 16S rDNA sequencing is available to clients who require sequence-based identification, but it can also identify unknown organisms for addition to the customized Bruker MBT Library.

Routine testing

The food microbiology routine testing laboratory analyzes, on average, 2,000-3,000 samples per day, but this can vary depending on the client requests. This laboratory is open seven days a week, 365 days a year, which contributes to the high volume of samples received. Prior to installing the MBT at Q Laboratories in 2017, the laboratory sub-contracted MALDI-TOF analyses for approximately 56 samples, most of the confirmations and identifications were done using conventional procedures, such as Gram staining and biochemical galleries. In 2017, approximately 4,200 confirmation and identification analyses were run with the MBT in Q Laboratories facilities. This coincides with a reduction in Gram stain volume, from 6,724 in 2016 to 5,292 the same year. Erin explains the services that this laboratory offers:

"We offer flexible options for turnaround time, to fit in with the client's needs, and adjust the pricing accordingly. This is something that our clients really value as it creates a completely customizable service. Many clients are willing to pay a premium for a next-day result, if they need a rapid pathogen identification, for example."

We can quite easily achieve a one day turnaround with the MBT. Using this system is now the most critical part of our business, because of the confidence in the results it provides."

Benefits of using the Bruker MALDI Biotyper (MBT) for microbial confirmation and identification as determined by Q Laboratories:

- Rapid, easy-to-use method with higher quality results than traditional biochemical methods.
- Comprehensive libraries result in fewer "no identification" results. Best option for high-throughput identification.
- Manual selection of area of the sample to analyse to counteract poor preparation.
- Multiple sample preparation methods to cater for different and difficult cultures to ensure accurate results for every organism type.
- Capacity to add organism IDs to custom library, to aid identification of unusual client-specific organisms.
- Longer period of time for testing or retesting culture plates. MBT can identify cultures that are 5-7 days old compared to Biochemical analysis which is restricted to a testing window of 24-48 hours
- Quicker time to results allows clients to make critical decisions faster.
- Reduced cost for diagnosis – up to a 50% reduction for some testing procedures

The laboratory uses a combination of different technologies for routine microbial confirmation and identification, and prior to purchasing the MBT, most often used biochemical-based methods taking.

With the MBT method, the time-to-result for accurate and reliable confirmations and identifications from isolated colonies on selective or non-selective culture plates can be reduced to between 30 minutes and two hours to investigate a full 96-spot target plate

(Bastin et al, J AOAC Int. 2018 Apr 27) [1,2]. Although Biochemical-based methods are a useful technique, several rounds of re-tests were often required due to reduced sensitivity, which limited the laboratory's time efficiency. Crowley comments on how introducing the MBT benefitted the laboratory workflow:

"Even when we had a final result with biochemical identity testing, we didn't have a high level of confidence in the result despite sometimes multiple retests. While biochemical methods can be beneficial in other ways, we really needed a solution that was more specific and sensitive, to provide the confidence we wanted for the final confirmation and identification result.

This, along with specific client requests of reliability, speed and confidence, drove the decision to acquire the Bruker MBT."



As Chief Scientific Officer, Crowley has played an integral role in modernizing the food microbiology laboratory, to streamline the workflow and make routine testing as efficient as possible. A recent evaluation found that 42 percent of the current work encompasses environmental *Listeria* testing, with some clients looking for a two day turnaround, and others requiring one day.

1 Bastin B, Bird P, Benzinger MJ Jr, Crowley E, Agin J, Goins D, Sohler D, Timke M, Shi G, Kostrzewa M. *Confirmation and Identification of Salmonella spp., Cronobacter spp., and Other Gram-Negative Organisms by the Bruker MALDI Biotyper Method: Collaborative Study, First Action* **2017.09**. J AOAC Int. 2018 Apr 27. DOI: <https://doi.org/10.5740/jaoacint.18-0012>

2 Bastin B, Bird P, Crowley E, Benzinger MJ Jr, Agin J, Goins D, Sohler D, Timke M, Awad M, Kostrzewa M. *Confirmation and Identification of Listeria monocytogenes, Listeria spp. and Other Gram-Positive Organisms by the Bruker MALDI Biotyper Method: Collaborative Study, First Action* **2017.10**. J AOAC Int. 2018 Apr 27. DOI: <https://doi.org/10.5740/jaoacint.18-0013>



The workflow

Clients typically submit their requests online, and the samples are either shipped or picked up by courier. Samples are brought into the distribution center, where they are barcoded and logged into the Laboratory Information Management (LIMS) system, which is automated, and tracks the sample ID, client information and test request. The request is instantly submitted to the microbiology food laboratory, where the operations team prepares the test batch and the analysts then carry out the tests.

Time-to-result depends on the type of test. Crowley expands on this:

“Organisms such as yeast or mold can take between three to five days, but if we’re looking at a pathogen test, clients most often request next day results.”

Typically for Salmonella or Listeria, it takes between 26-30 hours in total, from receipt of sample to the client receiving the results.”

Research and development – achieving AOAC certification

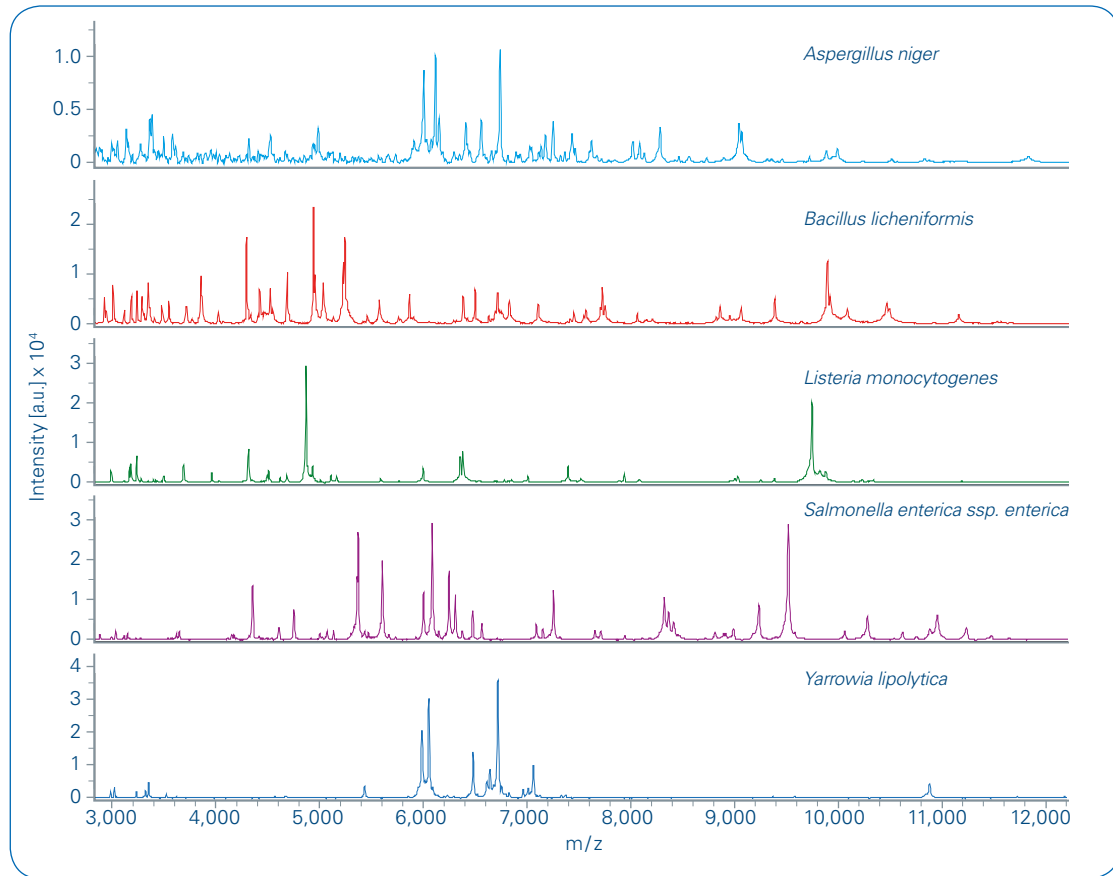
Whereas the client samples in the routine laboratory are food products, the ‘samples’ in the R&D laboratory are the technologies. Technology companies and diagnostic partners approach Q Laboratories with a new technology or assay for validation, for example for testing *Salmonella*, *Listeria* or *Legionella*, and request a proposal for the work. The first step is for the company to provide instructions for use, and Q Laboratories advise on the study design, which will be specific to the assay they are trying to market. Crowley explains the process:

“We spend a lot of time talking through the protocol with our clients and what the study design is going to look like. After that, with AOAC, there’s a consultant that will work with them on designing the protocol, preparing it, then working with us to get a quote. In order to be an independent laboratory with AOAC, all the information has to go through a consultant.”

The idea is to put the technology in the hands of a laboratory (or end user) which is not familiar with it, to test its robustness.”

From a European certification point of view, MicroVAL and AFNOR are the certification bodies, and the ‘Expert Laboratory’ (in this case, Q Laboratories) is the party that prepares the protocol, in collaboration with the technology provider. The Expert Laboratory then submits the protocol to the certification body for the technical reviewers to approve. Once approved, Q Laboratories starts the validation. The technology company usually supplies the proprietary reagents and kits, and Q Laboratories provides the materials needed for inoculation, and the reference method which the test has to be compared to.

“We provide periodic updates on the data to the client” explains Crowley, adding: “We let them know where we are up to with each phase of the study, so they know how their technology is performing. The most important factor in validation studies is being completely clear about the deadline. Some studies can take between three and five months to complete.”



Collaborative studies

In the case of an AOAC collaborative study or an ISO validation, a comparison study takes place with one in-house laboratory, such as that at Q Laboratories, which challenges the reproducibility of a technology. Crowley explains the process of such a study:

“As the leading laboratory, we are responsible for recruiting 12-15 laboratories throughout the world, which have agreed to volunteer to test the blind-coded samples we send them. All labs evaluate the samples using the same technology, on the same day. We have become very experienced at shipping dangerous samples abroad and adhering to each country’s strict regulations. Sometimes this is the most challenging aspect of the study. Once the analysis is complete, the labs submit the data for us to conduct the statistical analysis. We then prepare a journal-ready manuscript for the client and submit to a certification body, where the technical committee review and approve by

consensus. Approval means the method is fit for purpose, and meets the validation requirements. We conducted such a collaborative study with Bruker, the official methods study using the MBT.”

Bruker validation study

At the same time as acquiring the MBT in 2017, Q Laboratories began conversations with Bruker to initiate a validation study for this MALDI technology. By combining existing guidelines, such as the AOAC Appendix J guidelines and the new ISO 16140 part 6 standard [3], Q Laboratories and Bruker together created the most optimal study design to validate the use of MBT for food microbiology analysis. Crowley describes the validation process:

“Bruker were assessing the certification bodies for submission – AOAC for the US and MicroVAL for Europe – as there were a lot of limitations with the current guidelines.

The way the study designs for validating an identification method hadn't caught up with the newest technology, so this study really was the first of its kind."

Q Laboratories served as the lead laboratory for two separate studies, gaining AOAC-Official Methods of Analysis (OMA) status for the Bruker MBT in February 2018. The first was for confirmation and identification of *Salmonella* spp, *Cronobacter* spp and other Gram Negative organisms (OMA#2017.09), and the second was for the confirmation and identification of *Listeria monocytogenes*, *Listeria* spp and other Gram Positive organisms (OMA#2017.10) in food microbiology. Crowley explains how having OMA-approved technology benefits her laboratory:

"Having this certification really draws clients to Q Laboratories. In the food industry, there are specific approval procedures in place – having certification at the level of an AOAC Official Method, and an ISO validated/MicroVAL certified method, is acceptable to the Food and Drug Administration (FDA) for pathogen confirmation. They want a third party certification body to have approved the validation data, and deem that as an equivalent alternative confirmation method to their own methodology, which is considered the gold standard."

Until the OMA status was achieved for the MBT, Q Laboratories were unable to offer food clients confirmation services for pathogen contamination. The approval of the official method has allowed Q Laboratories to expand its identification laboratory, to more comfortably offer that option for food companies which are looking for finished product and raw material testing.

"Knowing that the MBT was going to be an OMA-approved method for pathogen confirmation in food was a big selling point for us, and a differentiator between other instruments"

explains Crowley, adding: *"We saw a return on investment on the MBT within the first year after purchase, with the biggest contributors factors being the 510K FDA approval for pharma testing and the OMA approval for food pathogen confirmation. This way it serves both segments of our microbiology work. "*

³ Together with the certification bodies, Q Laboratories and Bruker were pioneers in using the ISO 16140-part 6 technical rules for a validation study. The standard was almost of the final stage before publication.

Design and Results of the AOAC Collaborative Studies OMA 2017.09 & 2017.10

Target analyte	Confirmation and Identification rates	
	MBT	Reference methods*
<i>Salmonella</i> spp. Non <i>Salmonella</i> strains	100% 100%	99.6% 97.5% (87.5% correct ID)
<i>Cronobacter</i> spp. Non <i>Cronobacter</i> strains	100% 99.7%	95.5% 100% (93.8% correct ID)
<i>Listeria</i> spp. Non <i>Listeria</i> strains	100% (97.8% correct species ID) 98.9%	100% (86.5% correct species ID) 91.3%
<i>L. monocytogenes</i> Non <i>L. monocytogenes</i> strains	99.5% 99.3% (98.9% correct species ID)	87.1% 91.3%

² ≥ 14 collaborators from minimum 7 laboratories were involved per study. Each collaborator received minimum 16 target and 8 non-target strains.

When the confirmation rate and the identification rate differ, the identification rate is shown in brackets.

* ISO, FDA and USDA reference methods.

Using the MBT

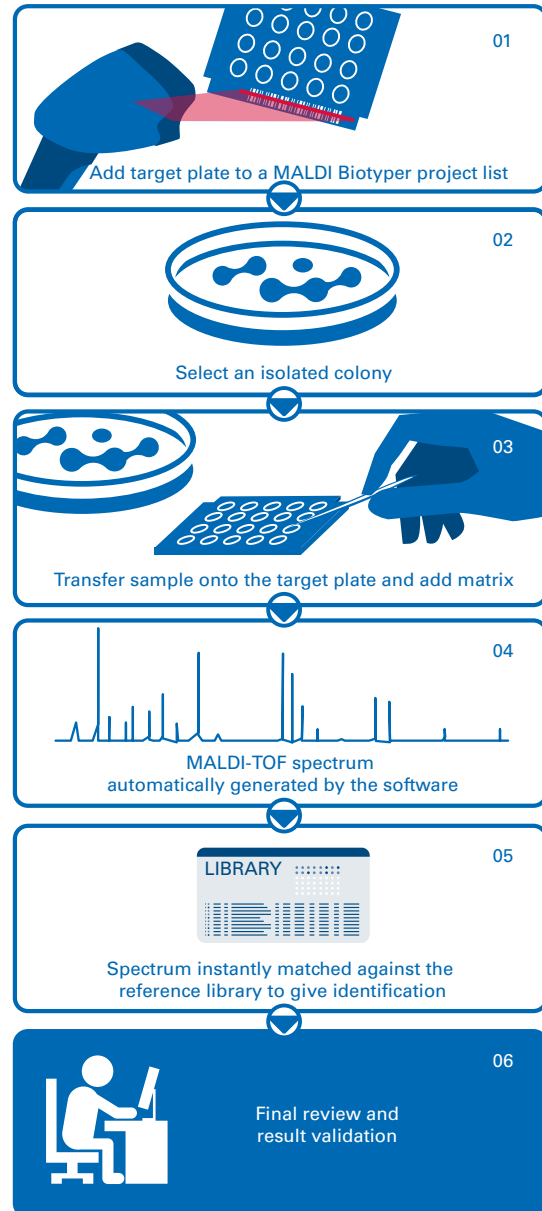
Q Laboratories have two MALDI Biotyper systems for food analysis: the MBT – used for routine analysis – and the MBT Smart, which was used alongside the MBT for Bruker’s validation study. This was to expand the validation scope, to cover both instruments.

Crowley comments on the use of the MBT in the microbiology food laboratory:

“When microbiologists are presented with mass spectrometry as a technology, which has historically been an analytical chemistry tool, it can be a little intimidating.

What has been most beneficial for us is how easy the MBT is to use. You don’t have to be a molecular biologist or an engineer to know how to run the technology.

There is a lot of robustness to the instrument, and we feel confident in training our analysts at every level to run it successfully. It only took about four weeks after installing the MBT to start running tests.”



Collaboration

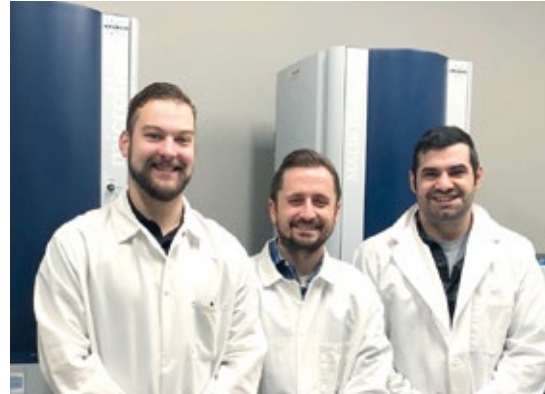
Q Laboratories works with a number of certification bodies, which have been critical for the growth of the company as an independent laboratory. It is the only North American laboratory to be an Expert Laboratory for all three of the major certification bodies: AOAC, MicroVAL and AFNOR. Crowley explains Q Laboratories' relationship with these bodies:

"Our commitment to being part of these certification bodies is developing our network of technical expertise that we can rely on for collaborating and expanding our opportunities for growth. Even simply running a question by them, and vice versa, is really beneficial. We are also actively involved with professional associations, such as the International Association of Food Protection. Our relationships with industry partners have been a valuable part of our business."

Collaboration is also prevalent amongst the laboratories within Q Laboratories. For example, the R&D laboratory will recommend a new technology it has just validated for certification, as a potential solution for the routine food and pharma microbiology laboratories. These routine laboratories will also reach out to the R&D team if there is a particular issue that a client has, to identify an alternative method. When Crowley became Chief Scientific Officer, she formed what is now known as the TAG team, and her team is primarily involved in taking the technology that the R&D laboratories are working with, helping to procure and validate them, then advertising to clients for routine use.

"It's a very interdisciplinary approach" comments Crowley, adding:

"It's an ongoing commitment to keeping our labs modern and meeting our client's needs."



Wesley Thompson, Daniel Barket and Benjamin Bastin, microbiologists at Q Laboratories with their MALDI Biotyper systems

Relationship with Bruker

Q Laboratories has an ongoing dialogue with Bruker, and has been involved in several symposia and poster presentations, presenting its work with the MBT validation. During the validation work, Q Laboratories reached out about routine work, and what could be improved in the existing laboratory, and Bruker is proactively making suggestions on how it can help. The relationship is moving forward as Bruker responds to the feedback it receives, as Crowley explains:

"All the must-haves are there. Where there are improvements to be made, Bruker responds quickly to ensure that we can continue to meet the demands of our customers."

For example, they expanded and updated the fungal library database, for identification of yeast and mold in the food market, which was a requirement we highlighted during our initial collaboration."

The ability to customize the database, based on the organisms that the laboratory has, greatly benefits the food microbiology routine laboratory.

"We have some very unique mold isolates or spoilage organisms, which are specific to particular customers" explains Crowley, continuing: "We then customize the database and store the isolates in our culture collection. You can then combine the Bruker identifications, and if you're repeatedly seeing the same organism, we can combine these into a challenge study on a product, for a client.

This builds a comprehensive picture of what challenges they might be facing, and builds a bridge between the routine and R&D laboratories."

Looking ahead

Q Laboratories is now working to grow its identification business, by meeting the needs of both food and pharma clients. This ties in with the company's strategy of also growing its environmental testing offering.

"Our drive to increase environmental testing nicely ties in with Bruker's capabilities, as the need for identification of environmental contaminants continues to rise" comments Crowley, adding:

"Being able to be part of the Bruker MBT validation study, something that we consider to be the first of its kind and quite revolutionary just in the study itself, was something that was really exciting to us as a company."

"We are also very honored to mention that the study design, the innovative technology and workflow, the breadth of method applicability, as well the recognized scope, are considered such an important achievement and improvement to food testing, ...

... that the method received the AOAC Award for the Best Method of the Year 2017-18."^[4]

4 The AOAC Official Methods of Analysis (OMA) Program sets standards for rigorous scientific scrutiny of methods to ensure that they demonstrate the highest level of confidence in analytical results. The AOAC Official Methods Board considers all methods that have been approved for First Action or Final Action OMA status within the last three years. Generally, some unique or particularly noteworthy aspect of the method is highlighted as making it worthy of the award, such as innovative technology or application, breadth of applicability, critical need, difficult analysis, and/or range of collaborators. The method demonstrates significant merit in scope or is an innovative approach to an analytical problem.

For more information on Q Laboratories, please visit <https://www.qlaboratories.com/>.

For more information on Bruker's MALDI Biotyper, please visit <https://www.bruker.com/products/mass-spectrometry-and-separations/maldi-biotyper-systems.html>

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About Q Laboratories

Q Laboratories has served the food, cosmetic, pharmaceutical, health and beauty care, and dietary supplement industries since 1966, offering comprehensive microbiology and chemistry laboratory and research and development services. An independent laboratory, combining state-of-the-art technology with personal service and attention, Q Laboratories can provide services to meet all testing and quality assurance needs. Q Laboratories is ISO/IEC 17025 Accredited, GMP/GLP Compliant, FDA Registered, a MicroVal Expert Laboratory, an AOAC Recommended Laboratory, An AFNOR Expert Laboratory, CDC ELITE Certified for Legionella Detection in Water, and Certified by the Ohio EPA for Potable Water Microbiological Analysis.

About Bruker Corporation

For more than 55 years, Bruker has enabled scientists to make breakthrough discoveries and develop new applications that improve the quality of human life. Bruker's high-performance scientific research instruments and high-value analytical solutions enable scientists to explore life and materials at molecular, cellular and microscopic levels.

In close cooperation with our customers, Bruker is enabling innovation, productivity and customer success in life science molecular research, in applied and pharma applications, in microscopy, nano-analysis and industrial applications, as well as in cell biology, preclinical imaging, clinical research, microbiology and molecular diagnostics..

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● **Bruker Daltonik GmbH**

Bremen • Germany
Phone +49 (0)421-2205-0

Bruker Scientific LLC

Billerica, MA • USA
Phone +1 (978) 663-3660

ms.sales.bdal@bruker.com - www.bruker.com/microbiology