Institution: Manchester Metropolitan University		
Unit of Assessment: A3 Allied Health Professions, Dentistry, Nursing and Pharmacy		
Title of case study: MALDI-TOF MS for microbe identification: the gold standard for clinical microbiologists worldwide		
Period when the underpinning research was undertaken: 2000 - 2007		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Valerie Edwards-Jones	Professor	1995 – 2014
Derek Gordon	Professor	1994 – 2002 (deceased)
Martin Claydon	Senior Lecturer	1994 – 2002 (deceased)
Diane Dare	Project Director	2000 – 2008 (retired)
Helen Bentley (née Sutton)	Research technician	2001 - present
Period when the claimed impact occurred: 1 August 2013 – 31 July 2020		
Is this case study continued from a case study submitted in 2014? Yes		

1. Summary of the impact

Pioneering research at Manchester Metropolitan University first demonstrated, then developed and validated, use of Matrix-Assisted Laser Desorption/Ionization-Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) for identification of microbial species and subtypes from whole cells. Two commercial systems underpinned by our research – Bruker's MALDI Biotyper and bioMérieux's VITEK MS – now dominate the global market. The number of systems in use worldwide has risen from approximately 1,200 in 2013 to over 7,200 in 2020. Accumulated revenues from new sales in this period are estimated at over USD1,600,000,000; cost savings from using MALDI-TOF MS identification surpass USD300,000,000 annually. Following FDA approval in late 2013, use of MALDI-TOF MS has become mainstream clinical microbiology practice; the UK's NHS and international clinical microbiology standards now recommend MALDI-TOF MS identification as standard for many infections. Peer-reviewed studies and head-to-head comparisons provide evidence of improved decision-making, patient benefit and better antibiotic stewardship due to faster diagnosis and more informed clinical decision-making.

2. Underpinning research

In the late 1990s, hospitals worldwide were experiencing a rapid rise in infections resistant to standard antimicrobial treatments, most notably from methicillin-resistant *Staphylococcus aureus* (MRSA). Clinical laboratories were in urgent need of new methods to identify pathological strains and their antibiotic susceptibility more quickly. A team of researchers from Manchester Metropolitan University was first to present data on the application of MALDI-TOF MS to identify bacterial species from intact cells in a matter of hours. The first proof-of-concept was published by our team in Nature Biotechnology in 1996. The research team went on to establish the basic instrumental parameters between 1997 and 1999, and a new linear TOF instrument, called M@LDI, was built by Micromass Ltd (now Waters Corporation) and delivered to the laboratory in April 2000. The research then focused on developing and validating the system for bacterial identification from intact cells.

The team realised that widespread adoption of MALDI-TOF MS would depend on a) access to a large library of high quality spectral data and b) robust validation and comparison of the technique against existing standard methods (culture-based or 16s rRNA ribosomal testing) using reference bacterial species. In 2000, Manchester Metropolitan spearheaded a collaboration between Micromass and the National Collection of Type Cultures (NCTC), part of the Public Health Laboratory Service. Each party analysed 20 NTCT reference strains per week in a blinded study with each strain analysed 12 times at each site. In the first year, the project produced 36,000 spectra, which formed the basis of composite reference spectra. The system was challenged with isolates of unknown and clinical origin with high correlation between spectrum matches across all three laboratories. The research effort established and validated a reference spectral library for 118 genera (382 species). At this early stage, successful identification of wild type strain varied from 30% to 100%, depending on the level of representation of the species within the database **[1]**.

An early study also measured intra- and inter-laboratory reproducibility of results between Manchester Metropolitan and the Colindale Public Health Laboratory. It showed that >75% of spectra peaks remained constant between replicate experiments and >60% peaks were

constant for replicate analyses conducted in different locations. The study highlighted the importance of following standard protocols; it also revealed which culture media worked best for MALDI-TOF MS analysis **[2]**.

Collaborative research continued through to 2007. In one study, the researchers investigated 95 clinical isolates from the Royal London Hospital and 39 isolates from the Health Protection Agency's Staphylococcal Reference Unit. They used the existing database library of more than 5,000 profiles of various bacterial pathogens to identify correctly all isolates to the correct genus and species level (with additional purification for just four isolates), confirmed by collaborating institutions using standard 16s rRNA ribosomal testing. The researchers concluded that "MALDI-TOF-MS has the potential to perform high-throughput identification of clinical isolates of *S. aureus* despite the inherent diversity of this species" **[3]**. A later collaboration brought the team's expertise to researchers in Belgium, and Berlin-based AnagnosTec. This study successfully demonstrated rapid and accurate identification of *Burkholderia cepacia* complex, which is important clinically for patients with cystic fibrosis **[4]**.

Alongside this intense activity to create the world's first clinically-relevant database for MALDI-TOF MS bacterial identification, Professor Edwards-Jones was first to hypothesise that the technique may also be able to distinguish between some species subtypes and functional variants. She suggested that MALDI-TOF MS might provide rapid distinction between methicillin-resistant and susceptible *S. aureus*.

She led the first proof-of-concept study on MALDI-TOF MS as an alternative for antibiotic susceptibility testing in 2000 **[5]**. She then focused her research on methodological and protocol optimisation, highlighting that reproducibility – which dogged subsequent research efforts in the field – would require further work, and would certainly only be possible "if variable parameters such as sample preparation, media, growth condition, etc. are standardised" **[6]**. These studies effectively sparked a search for the 'holy grail' of rapid susceptibility testing that continues to shape the development of MALDFI-TOF MS in clinical microbiology to this day.

3. References to the research

Note: Citations, Web of Science (citations versus expected citations), January 2021

1. Keys CJ, **Dare** DJ, **Sutton** H, Wells G, Lunt M, McKenna T, McDowall M, Shah HN (2004). Compilation of a MALDI-TOF mass spectral database for the rapid screening and characterisation of bacteria implicated in human infectious diseases. Infection, Genetics and Evolution: J. Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases. 4(3):221-242. DOI: 10.1016/j.meegid.2004.02.004. *Citations: 126 (expected 44.81)*.
2. Walker J, Fox AJ, **Edwards-Jones** V, **Gordon** DB (2002). Intact cell mass spectrometry (ICMS) used to type methicillin-resistant *Staphylococcus aureus*: media effects and inter-laboratory reproducibility. J. Microbiol. Methods 48:117-126. DOI: 10.1016/s0167-7012(01)00316-5. *Citations: 116 (expected 36.32)*.
3. Rajakaruna L, Hallas G, Molenaar L, **Dare** D, **Sutton** H, Encheva V, Culak R, Innes I, Ball G, Sefton AM, Eydmann M, Kearns AM, Shah HN (2009). High-throughput identification of clinical isolates of *Staphylococcus aureus* using MALDI-TOF-MS of intact cells. Infection, Genetics and Evolution 9(4):507-513. DOI: 10.1016/j.meegid.2009.01.012. *Citations: 43 (expected 22.51)*.
4. Vanlaere E, Sergeant K, Dawyndt P, Kallow W, Erhard M, **Sutton** H, **Dare** D, Devreese B, Samyn B, Vandamme P (2008). Matrix-assisted laser desorption ionisation-time-of-flight mass spectrometry of intact cells allows rapid identification of *Burkholderia cepacia* complex. J. Microbiol. Methods 75(2):279-286. DOI: 10.1016/j.mimet.2008.06.016. *Citations: 65 (expected 29.48)*.
5. **Edwards-Jones** V, **Claydon** MA, Evason DJ, Walker J, Fox, AJ, **Gordon**, DB (2000). Rapid discrimination between methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* by intact cell mass spectrometry. J. Med. Microbiol. 49(3):295-300. DOI: 10.1099/0022-1317-49-3-295. *Citations: 179 (expected 50.68)*.
6. Jackson KA, **Edwards-Jones** V, Sutton CW, Fox AJ (2005). Optimisation of intact cell MALDI method for fingerprinting of methicillin-resistant *Staphylococcus aureus*. J. Microbiol. Methods 62(3):273-84. DOI: 10.1016/j.mimet.2005.04.015. *Citations: 70 (expected 42.37)*.

Funding

G1. Joint CPHL/Withington Hospital Studentship. Public Health Laboratory Services (Project No. F-00031). 2002-2005. GBP3,862. PI: Edwards-Jones.

G2. Meningococcal Sequence Typing Project. Public Health Laboratory Services (Project No. F-00138). 2002-2007. GBP12,595. PI: Edwards-Jones.

Indicators of research quality

- Reference [1] is cited in four patent family applications (Plum Metrics) including patents assigned to bioMérieux and Bruker (see Section 4).
- Reference [5] is cited in six patent family applications (Plum Metrics) including patents assigned to bioMérieux and Bruker (see Section 4).
- Reference [5] is the earliest paper cited in subsequent studies (including industry-sponsored work) investigating MALDI-TOF MS for bacterial subtyping and AST (see Section 4).

4. Details of the impact

In REF2014 we established how our research underpinned the eventual commercialisation of MALDI-TOF MS for bacterial identification. Our work influenced commercial development through three distinct pathways. First, we published the earliest proof-of-concept studies (references [1] and [5]); these papers proved seminal to subsequent academic studies and commercial R&D. Second, the research provided the methodological scaffold that companies were then able to refine, develop and patent (see Section 3). Third, our methods, learning and techniques were transferred into industry through the collaboration with AnagnosTec, which specialised in MALDI-TOF MS bacterial identification and was acquired by bioMérieux in 2010.

In REF2014 we described significant clinical, operational and economic benefits arising from early adoption 2008-2013. Here we describe how this MALDI-TOF MS 'revolution' is now embedded as a routine, gold standard technique in clinical, research and analytical microbiology worldwide. System sales since 2013 have driven growth and steered business strategy for the two global market leaders: Bruker and bioMérieux. We also show that the pioneering work of Edwards-Jones initiated worldwide academic and industry R&D that, since 2013, has underpinned significant impact in MALDI-TOF MS-based antibiotics susceptibility testing (AST). Finally, we evidence how adoption of MALDI-TOF MS continues to improve patient outcomes and antibiotic stewardship.

Commercial impact: global sales, business growth and strategic focus

In 2013 there were approximately 1,200 MALDI-TOF MS systems installed worldwide (800 Bruker Biotyper; 400 bioMérieux VITEK MS) **[A]**. By 2020 Bruker had installed approximately 3,800 systems worldwide (3,000 new installations since 2013) **[A].** Assuming similar sales for bioMérieux (data not publicly available), the global installed base in 2020 was approximately 7,200 systems making upward of 300,000,000 microbial identifications per year **[A]**.

Taking a fixed unit price of USD270,000 (03-2015) and USD30,000 (03-2015) for an annual service and maintenance contract **[B]**, sales of new systems since 2013 have generated revenue of approximately USD1,620,000,000 (03-2015), unadjusted for inflation. Servicing contracts from the entire installed base generated approximately USD250,000,000 (07-2020) in 2020, assuming 3% annual inflation. As an indicator of the significance to Bruker, approximate 2019 sales of 400 new Biotyper systems, worth USD108,000,000 (03-2015) plus 3,400 annual service contracts, worth USD118,245,000 (07-2020), constituted 18.2% of annual revenue for Bruker's BSI Life Science segment (2019 annual figures) and a growth driver **[C]**.

MALDI-TOF MS has driven growth for both companies. In 2016, Bruker's President and CEO described Biotyper as a *"key profitable growth platform"* while bioMérieux reported that VITEK MS showed *"double digit growth"* compared to just 3% overall growth for microbiology sales and 7.1% overall annual company sales growth **[D]**.

The commercial success of the MALDI-TOF systems has been a cornerstone of both companies' strategy. To illustrate, in response to intense market demand, Bruker has enjoyed a succession of product launches and acquisitions to bolster Biotyper's sub-typing capabilities and leverage the platform for AST (as first explored by Edwards-Jones). Since 2013, Bruker has launched: a beta lactamase module for selective antibiotic testing (2014); the Sepsityper kit for bacterial identification from positive blood culture (2015); a specific subtyping module for PSM-mec-positive MRSA and cfiA-positive *Bacteroides fragilis* strains (2016); the first MS-based AST for non-clinical detection of carbapenem-resistant *Klebsiella pneumonia* (2017); and the MERLIN system for phenotypic growth inhibition detection (2017) **[E]**.

In 2019, Bruker launched its updated Biotyper Sirius for *"near-universal bacterial identification"* with a fast and robust assay to detect colistin-resistant bacteria. Bruker's overarching strategy to expand its total addressable market emphasises the importance of products for fast microbe identification. It has continuously developed its MALDI-TOF capability or acquired new products that add selected AST to the standard Biotyper workflow **[A,E]**.

This intense focus on AST capabilities can trace its origins back to the early research by Edwards-Jones (see [5-6], above). The key study demonstrating MALDI-TOF MS detection of PSM-mec-positive *S. aureus* (2014) and a recent 2020 breakthrough announced by Bruker scientists on the feasibility and accuracy of a MALDI-TOF MS-based growth assay for rapid AST of *S. aureus* reveal a sustained, demand-led industrial R&D focus on MALDI-TOF MS for AST. These subtyping and AST breakthroughs both acknowledge Edwards-Jones' first conceptualisation of MALDI-TOF MS for strain susceptibility subtyping **[F]**.

Changing clinical practice: FDA approval makes MALDI-TOF MS bacterial identification standard clinical microbiology practice

Manchester Metropolitan researchers were quick to realise the clinical potential of the MALDI-TOF MS technique but emphasised the need for standardisation in sample preparation, media selection and protocols to assure accuracy, and to minimise the risk of false negative results (see [2], above).

The FDA approved VITEK MS and Biotyper for clinical diagnostics in August and November 2013, respectively, opening up the US market for accelerated uptake and sales growth **[G]**. Updates to the clinical reference spectra libraries have received several subsequent approvals, including for the bacteria Brucella and the fungus *Candida auris,* an emerging pathogen responsible for severe bloodstream infections but often misidentified using culture assays, leading to inappropriate treatment. It was made notifiable at the 2018 Council for State and Territorial Epidemiologists in the US in 2018 **[G]**.

Use of MALDI-TOF MS for microbial identification is now accepted among standard diagnostic procedures for clinical laboratories. For example, between 2014 and 2019 Public Health England (PHE) published updates to its Standards for Microbiology Infections, compiled and agreed between a wide range of stakeholders including the NHS, the Royal College of Pathologists and approximately 15 other professional organisations and learned societies. All NHS clinical laboratories are expected to adhere to these guidelines. 16 out of 35 extant bacteriology standards and all 26 extant identification standards now include MALDI-TOF MS for identification and subtyping in some cases. In 2016 PHE also published SMI TP40 to instruct clinical microbiology laboratories on standard protocols for MALDI TOF MS test procedures **[H]**. The Clinical Laboratory Standards Institute also published its standard 'Methods for the Identification of Cultured Microorganisms Using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry'. The organisation has more than 1,600 member organisations worldwide that collaborate to establish and adopt evidence-based standards and best practice guidelines for clinical laboratories **[I]**.

Time and cost savings and improving clinical outcomes

Numerous academic studies have documented impressive time and cost savings from the use of MALDI-TOF compared to traditional culture techniques. For example, a clinical microbiology laboratory in a large 800-bed hospital in the US reported annual cost savings of approximately USD74,000 (03-2015), approximately 52% of the cost of identification from cultures **[B]**. Assuming that just half of global-installed MALDI-TOF systems are used in similar high-throughput clinical laboratories, the global cost savings would be approximately USD309,000,000 (03-2020) per year (adjusted for 3% annual inflation)**.** Evidence of substantial time savings by reducing clinical identification times from >48 hours for culture methods to less than 24 hours (and sometimes as little as one hour) for MALDI-TOF MS are now well established, with the capital cost of the instrument offset in approximately three years **[B].**

Academic studies have also evaluated clinical impacts arising from the application of the MALDI-TOF technique, based on the premise that faster and more accurate identification of pathogens enables more rapid antibiotic choices/treatment decisions and hence better treatment outcomes. One study found conclusive evidence that *"faster identification using MALDI-ToF assists the clinician in assessing the significance of a blood culture isolate on day one. It can allow earlier appropriate choice of antimicrobial agent..."* **[J]**. A 2018 systematic literature review of the impact of MALDI-TOF MS in public health and hospital hygiene provides robust evidence

of significant clinical benefits arising from MALDI-TOF MS identification including high accuracy and optimal bacteriaemia treatment within 48 hours and antibiotic stewardship through AST. The authors conclude: *"The increase in speed of pathogen detection enables improvement of antimicrobial therapy, infection prevention and control measures leading to positive impact on public health. For antibiotic susceptibility testing and bacterial typing, it represents a rapid alternative to time-consuming conventional techniques... Taking into account the great impact of MALDI-TOF MS during the past 10 years, the knowledge that has been acquired during this time and the great flexibility of the technique, we think that its influence in public health will only become bigger in the coming years"* **[J]**. In 2016 The Centers for Disease Control's MicrobeNet service launched its MALDI-TOF Module in partnership with Bruker, with a primary mission to boost species representation for less common and difficult to identify bacterial and fungal species. This helped increase MicrobeNet users from approximately 700 in 2016 to over 2,200 in 2019, representing over 1,000 organizations worldwide. MALDI-TOF is responsible for nearly 90% of the 30,000 identifications through MicrobeNet each year. MicrobeNet has been critical for rapid triage of *Elizabethkingia anophelis* outbreak isolates as well as the global response to *Candida auris* **[J]**.

Beyond healthcare

Despite a heavy focus on clinical applications, more recently MALDI-TOF bacterial identification has begun to extend its reach into other fields, especially environmental and marine microbiology, and food and consumer product safety and quality control. For example, in 2018 Bruker's Biotyper received approval from AOAC International Approvals for two official methods of analysis in food microbiology (AOAC 2017.09 and AOAC 2017.10) that were later awarded the body's 'Method of the Year' **[E]**. AOAC International "brings together government, industry, and academia to establish standard methods of analysis that ensure the safety and integrity of foods and other products that impact public health around the world."

5. Sources to corroborate the impact

A. REF2014 impact case study and a Bruker corporate presentation (2020) *provide evidence of growth in the global installed base of the Biotyper MALDI-TOF MS system and the annual number of bacterial identifications worldwide in 2020. Note: Bruker and bioMérieux are acknowledged as the entrenched market leaders in the field; bioMérieux has not disclosed sales volumes, but it is reasonable to assume volumes similar to those of Bruker.*
B. Tran *et al.* (2015). http://doi.org/10.1128/JCM.00833-15 *provides evidence on the capital outlay and servicing cost of VITEK MS system (2015 prices).*
C. Bruker Corporation Annual Report 2019 *provides evidence of 2019 financial performance and statements on contribution of life science mass spectrometry and microbiology products to growth*.
D. Bruker Q3 2015 Financial Results and bioMérieux Annual Report 2015 *provide evidence of the contribution of Biotyper and VITEK MS to commercial growth.*
E. A portfolio of Bruker press releases (2014-2019) *provides evidence of Bruker's strategic sub-typing and AST technology acquisitions and product launches, alongside announcements on official methods for use of the Biotyper platform for food microbiology.*
F. Portfolio of three peer-reviewed papers demonstrates progress in the use of MALDI-TOF MS for direct identification of antibiotic resistant strains of *S. aureus*. *The evidence highlights the pioneering research of Edwards-Jones as the origin of subsequent development of MALDI-TOF MS-based subtyping and AST.*
G. Bruker and bioMérieux press releases and web content *provide evidence of FDA approvals.*
H. An analysis of the SMI library *indicates which standards permit the use of MALDI-TOF MS*.
I. Extract from the Clinical Laboratory Standards Institute standard: "Methods for the Identification of Cultured Microorganisms Using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry".
J. French *et al.* (2016) https://doi.org/10.1371/journal.pone.0169332 and Rodríguez-Sánchez *et al.* (2019) https://doi.org/10.2807/1560-7917.ES.2019.24.4.1800193 *provide evidence of improved clinical outcomes in a hospital setting and for public health.* Information from the CDC MicrobeNet *provides evidence of growth in usage and role in rare infection outbreaks.*