SINGER INSTRUMENTS
A RESPONSIBILITY TO SCIENCE!

CARDINAL

Automating MALDI-TOF sample preparation protocols to facilitate bacterial identification.

Automating MALDI-TOF sample preparation protocols to facilitate bacterial identification

APPLICATION

Michael D. Nairn[1], Philip Kirk[2], Matthew E. Openshaw[1], Oliver Severn[2], Leah Ashley[2]

1, Shimadzu, Manchester, UK; 2, Singer Instruments, Roadwater, UK

ABSTRACT

Matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) is a mass spectrometry (MS) that characterises various intact biomolecules. In microbiology, MALDI-TOF aids pathogen detection and microbial profiling by identifying bacteria to the strain level. However, MALDI-TOF requires rigorous sample processing methods to ensure the reproducibility of bacterial cells from MS data. In this application, we show that introducing automation into MALDI-TOF pipelines can reduce sample processing times while maintaining accurate bacterial identification. This document provides you with the blueprint to adopt automation into your MALDI-TOF pipelines with the PIXL colony picker.

Identifying bacteria at the species level underpins clinical and environmental microbiology. The introduction of MALDI-TOF has greatly increased the resolution in bacterial profiling, largely replacing more traditional qualitative staining assays for characterising bacteria. In the clinic, MALDI-TOF is helping diagnose disease[1] and identify antibiotic-resistant bacteria[2]. Microbiologists also use MALDI-TOF to distinguish bacterial strains and analyse human and environmental microbiomes[3,4]. MALDI-TOF's success in conducting species-level bacterial identification lies in profiling the proteins that a microorganism produces[6]. Different bacterial species have unique protein signatures, from their ribosomal proteins to membrane proteins, that MALDI-TOF can distinguish.

Although MALDI-TOF can recognise bacterial species by its proteins, generating reproducible MS data for bacterial identification requires many steps. Variables within these steps, such as the sample preparation method, the amount of bacterial colony obtained, and the age of the colony, can affect the quality of the MS data[7]. With many experimental sources of variation present in MALDI-TOF, researchers have increasingly looked to automation to minimise technical errors.

THE QUESTION: AUTOMATION AND MALDI-TOF

Integrating automation provides several benefits for researchers. Most notably, automating experimental workflows reduces human errors, increases reproducibility, and enhances researcher efficiency[8]. For MALDI-TOF MS workflows, automating the sample processing and species identification protocols could make species-level calls more reproducible and accurate. In this application, we investigated whether the colony sample preparation procedure could be automated for MALDI-TOF MS species identification.

METHODS

An automated MALDI-TOF pipeline was developed to streamline bacterial identification from lab cultures (Figure 1).

This pipeline was tested on *Escherichia coli* for method preparation and *Bacillus subtilis* (*B. subtilis*), *Cellulomonas uda* (*C. uda*), *and Pantoea agglomerans* (*P. agglomerans*) for testing the automated method. In the automated pipeline, colonies were picked from cultured samples and deposited onto a disposable MALDI MS sample plate, such as the FlexiMass-DS slides (Shimadzu), using a PIXL precision microbial colony picker (Singer Instruments). The automated protocol was compared with manual preparations where an inoculation loop was used to spot the plates with sample material. The automation protocol was then further refined by determining whether it should feature a pinning method or a smearing method (Figure 2).

To further evaluate the automated pipeline, different areas of the culture plates were also picked for each bacterial species (Figure 3).

Once the samples were prepared, the pins and smears were analysed on an iDplus Performance MALDI-TOF mass spectrometer (Shimadzu) and submitted to a SARAMIS database for identification.

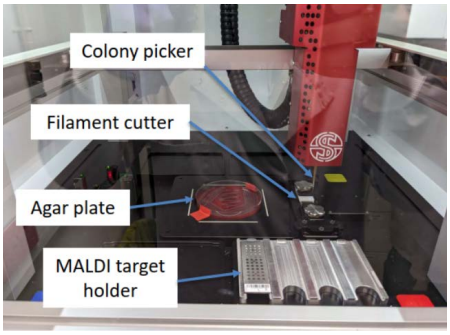

Figure 1. The PIXL colony picking robot [left] and its inner components [above], the automated system used for MALDI-TOF sample processing.

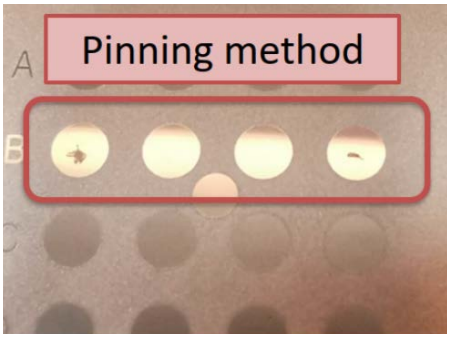

Figure 2. The two automated bacterial transfer methods employed. The pin method (far left) 'dabs' the picked sample onto a spot on the target surface, while the smear method (left) forms a smear across the target surface.

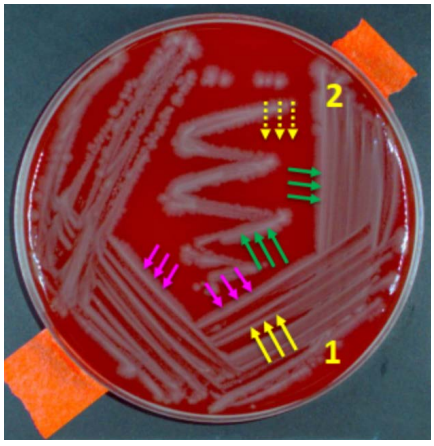

Figure 3. Three areas where bacteria were picked. Green refers to the thickest part of the culture growth, or "Deep". Pink represents the "Edges" of the culture growth streak. Yellow refers to the material picked from the thickest part of the culture growth 'steak' (solid arrows, 1) then dabbed onto an area of agar not containing any culture to remove excess material prior to depositing onto the MALDI target (dashed arrows, 2).

RESULTS

To begin assessing the automated sampling method for MALDI-TOF, researchers tested it on three types of colonies across the culture plates. Doing so showed that the automated smear and automated single/double deposition of matrix solution were equally accurate in identifying *E. coli* with >99.9% confidence (Figure 4). In contrast, the manual method introduced plasticisers and other contaminants that obfuscated the *m/z* peaks for identifying microbial species.

With the automated protocol developed, researchers then determined whether the automated sample collection could identify three other bacterial species with high confidence. The automated protocol allowed two of the species, *P. agglomerans* and *C. uda*, to be identified with over 99.9% confidence. While there were red IDs for the *B. subtilis* identification, this was due to a mixed ID in the database, not the automated sampling method itself. Nonetheless, most promising about the data was the MALDI-TOF MS spectra for *P. agglomerans* being nearly identical for both the PIXL automation and manual approaches (Figure 6).

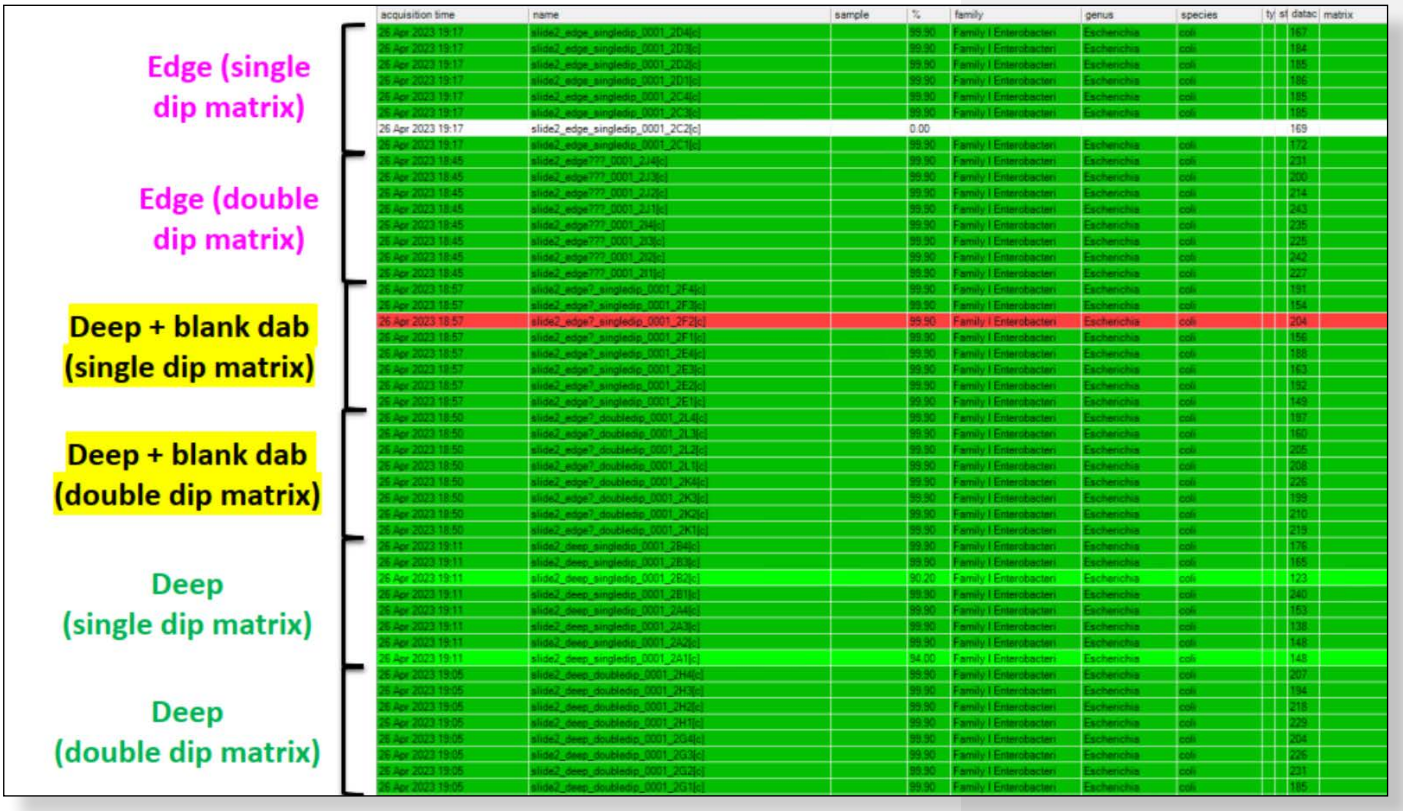

Figure 4. Identification results for *E. coli* samples prepared with the automated smear pipeline for samples taken from all areas of the culture plate. Dark green = >99.9% confidence, light green = 90.0 – 99.8% confidence, red = mixed ID, white = no ID.

27 Apr 2023 18:03	slide2_Pagg_doubledip_0001_2L4[c]		99.90	Family I Enterobacteri	Pantoea	agglomerans			222
27 Apr 2023 18:03	slide2_Pagg_doubledip_0001_2L3[c]		99.90	Family I Enterobacteri	Pantoea	agglomerans			204
27 Apr 2023 18:03	slide2_Pagg_doubledip_0001_2L2[c]		99.90	Family I Enterobacteri	Pantoea	agglomerans			214
27 Apr 2023 18:03	slide2_Pagg_doubledip_0001_2L1[c]		95.00	Family I Enterobacteri	Pantoea	agglomerans			214
27 Apr 2023 18:03	slide2_Pagg_doubledip_0001_2K4[c]		99.90	Family I Enterobacteri	Pantoea	agglomerans			218
27 Apr 2023 18:03	slide2_Pagg_doubledip_0001_2K3[c]		99.90	Family I Enterobacteri	Pantoea	agglomerans			217
27 Apr 2023 18:03	slide2_Pagg_doubledip_0001_2K2[c]		99.90	Family I Enterobacteri	Pantoea	agglomerans			226
27 Apr 2023 18:03	slide2_Pagg_doubledip_0001_2K1[c]		99.90	Family I Enterobacteri	Pantoea	agglomerans			202
27 Apr 2023 18:03	slide2_Pagg_doubledip_0001_2J4[c]		99.90	Family I Enterobacteri	Pantoea	agglomerans			175
27 Apr 2023 18:03	slide2_Pagg_doubledip_0001_2J3[c]		99.90	Family I Enterobacteri	Pantoea	agglomerans			194
27 Apr 2023 18:03	slide2_Pagg_doubledip_0001_2J2[c]		98.60	Family I Enterobacteri	Pantoea	agglomerans			153
27 Apr 2023 18:03	slide2_Pagg_doubledip_0001_2J1[c]		99.90	Family I Enterobacteri	Pantoea	agglomerans			226
27 Apr 2023 18:03	slide2_Pagg_doubledip_0001_2I4[c]		95.20	Family I Enterobacteri	Pantoea	agglomerans			180
27 Apr 2023 18:03	slide2_Pagg_doubledip_0001_2I3[c]		95.20	Family I Enterobacteri	Pantoea	agglomerans			189
27 Apr 2023 18:03	slide2_Pagg_doubledip_0001_2I2[c]		99.90	Family I Enterobacteri	Pantoea	agglomerans			157
27 Apr 2023 18:03	slide2_Pagg_doubledip_0001_2I1[c]		91.80	Family I Enterobacteri	Pantoea	agglomerans			147
27 Apr 2023 18:19	slide2_Cuda_doubledip_0001_2H4[c]		99.90	Family VI Cellulomonod	Cellulomonas	uda			168
27 Apr 2023 18:19	slide2_Cuda_doubledip_0001_2H3[c]		99.90	Family VI Cellulomonod	Cellulomonas	uda			197
27 Apr 2023 18:19	slide2_Cuda_doubledip_0001_2H2[c]		99.90	Family VI Cellulomonod	Cellulomonas	uda			206
27 Apr 2023 18:19	slide2_Cuda_doubledip_0001_2H1[c]		99.90	Family VI Cellulomonod	Cellulomonas	uda			187
27 Apr 2023 18:19	slide2_Cuda_doubledip_0001_2G4[c]		99.90	Family VI Cellulomonod	Cellulomonas	uda			178
27 Apr 2023 18:19	slide2_Cuda_doubledip_0001_2G3[c]		99.90	Family VI Cellulomonod	Cellulomonas	uda			186
27 Apr 2023 18:19	slide2_Cuda_doubledip_0001_2G2[c]		99.90	Family VI Cellulomonod	Cellulomonas	uda			175
27 Apr 2023 18:19	slide2_Cuda_doubledip_0001_2G1[c]		99.90	Family VI Cellulomonod	Cellulomonas	uda			178
27 Apr 2023 18:19	slide2_Cuda_doubledip_0001_2F4[c]		99.90	Family VI Cellulomonod	Cellulomonas	uda			155
27 Apr 2023 18:19	slide2_Cuda_doubledip_0001_2F3[c]		99.90	Family VI Cellulomonod	Cellulomonas	uda			166
27 Apr 2023 18:19	slide2_Cuda_doubledip_0001_2F2[c]		99.90	Family VI Cellulomonod	Cellulomonas	uda			190
27 Apr 2023 18:19	slide2_Cuda_doubledip_0001_2F1[c]		99.90	Family VI Cellulomonod	Cellulomonas	uda			189
27 Apr 2023 18:19	slide2_Cuda_doubledip_0001_2E4[c]		99.90	Family VI Cellulomonod	Cellulomonas	uda			181
27 Apr 2023 18:19	slide2_Cuda_doubledip_0001_2E3[c]		99.90	Family VI Cellulomonod	Cellulomonas	uda			179
27 Apr 2023 18:19	slide2_Cuda_doubledip_0001_2E2[c]		99.90	Family VI Cellulomonod	Cellulomonas	uda			154
27 Apr 2023 18:19	slide2_Cuda_doubledip_0001_2E1[c]		99.90	Family VI Cellulomonod	Cellulomonas	uda			159
27 Apr 2023 18:29	slide2_Bsubt_doubledip_0001_2D4[c]		99.90	Family I Bacillaceae	Bacillus	subtilis			171
27 Apr 2023 18:29	slide2_Bsubt_doubledip_0001_2D3[c]		99.90	Family I Bacillaceae	Bacillus	subtilis			155

Figure 5. Identification results for the bacterial species *B. subtilis*, *C. uda*, *and P. agglomerans* when using the optimised automated method. Dark green = >99.9% confidence, light green = 90.0 – 99.8% confidence, red = mixed ID.

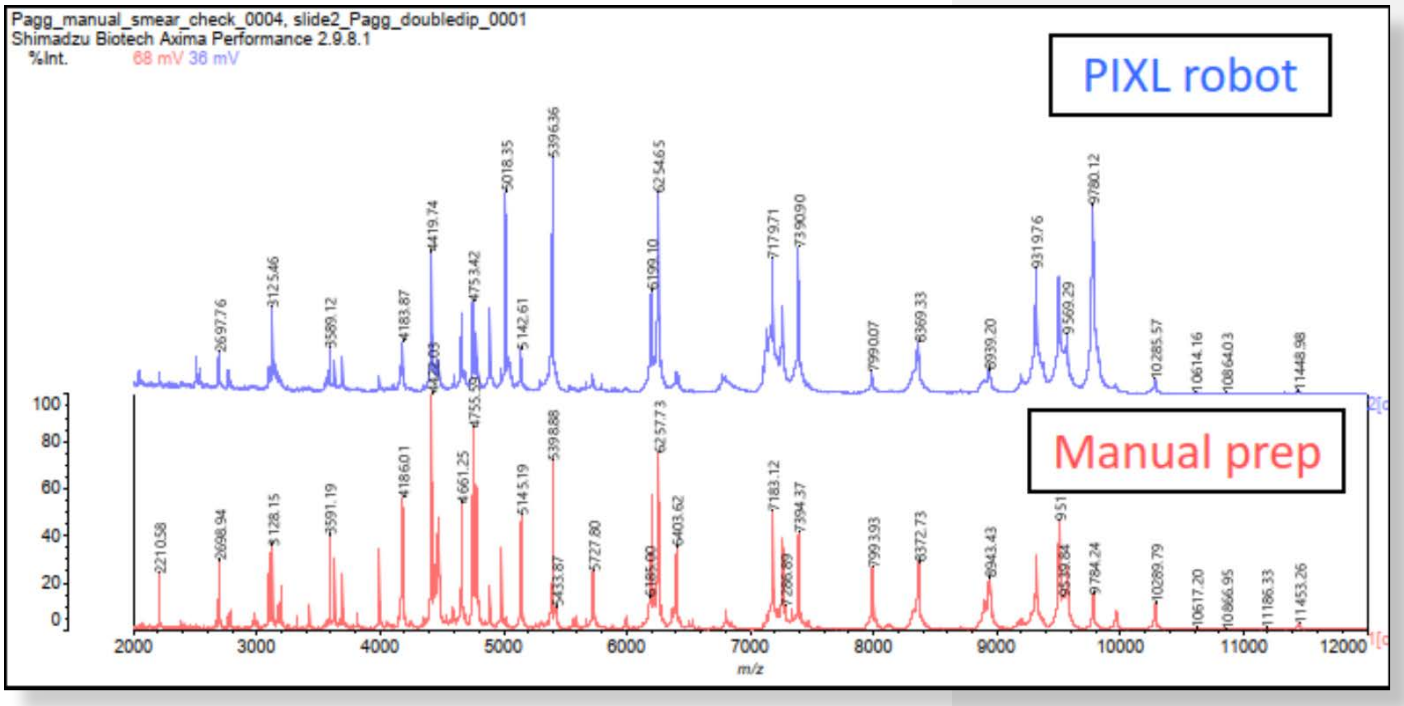

Figure 6. Representative MALDI-TOF MS spectra for *P. agglomerans* with a manually prepared smear sample (in red) and a smear sample prepared with the automated protocol (in blue).

CONCLUSIONS

Integrating automation into research pipelines can streamline research protocols and improve reproducibility in biomedical research. In microbiology, automation may also prove useful for identifying microbial species with MALDI-TOF. Here, we show that automation can facilitate sample collection and streaking protocols, both of which affect the accuracy and reproducibility of MALDI-TOF MS-based bacterial identification.

Using the PIXL to automate colony picking and matrix deposition allowed reference microbial species to be identified correctly and reproducibly. Furthermore, the automated colony-picking method generated MALDI-TOF MS spectra like those produced with the manual method. Put together, automating the sample collection protocol makes MALDI-TOF identification more reproducible.

With automated MALDI-TOF MS pipelines now possible, researchers can efficiently type and characterise microbes for a wide range of applications, from pathogen identification to antibiotic sensitivity testing.

Contact us at contact@singerinstruments.com to learn more about the PIXL colony picker and how it can be seamlessly integrated into your bacterial identification workflows.

REFFERENCES

1. Patel, R. (2015) MALDI-TOF MS for the Diagnosis of Infectious Diseases. Clinical Chemistry, 61(1), pp. 100-111. doi: 10.1373/clinchem.2014.221770.

2. Kostrzewa, M., Sparbier, K., Maier, T., & Schubert, S. (2013) MALDI-TOF MS: an upcoming tool for rapid detection of antibiotic resistance in microorganisms. PROTEOMICS – Clinical Applications, 7(11-12), pp. 767-778. doi: 10.1002/prca.201300042.

3. Mojumdar, A., Yoo, H. J., Kim, D. H., & Cho, K. (2022) High-Throughput Screening Technique for Microbiome using MALDI-TOF Mass Spectrometry: A Review. Mass Spectrometry Letters, 13(4), pp. 106-114.

4. Ashfaq, M. Y., Da'na, D. A., & Al-Ghouti, M. A. (2022) Application of MALDI-TOF MS for identification of environmental bacteria: A review. Journal of Environmental Management, 305, 114359. doi: 10.1016/j.jenvman.2021.114359.

SINGER INSTRUMENTS

A RESPONSIBILITY TO SCIENCE!

Roadwater
Watchet
Somerset
TA23 0RE, UK.

+44 (0)1984 640226 (tel)
+44 (0)1984 641166 (fax)

contact@singerinstruments.com
singerinstruments.com

Scan to visit the PIXL webpage.

OUR DISTRIBUTORS

For a list of our distributors visit:
singerinstruments.com/friends

/SingerInstruments