LABORATORY PROTOCOL

Identification of bacteria with MALDI-TOF (Matrix-Assisted Laser Desorption-Ionization Time Of Flight)

April 2026
Version 1

HISTORY OF CHANGES				
Version	Sections changed	Description of change	Date	Approval
1	New document	Developed by Dafni K. Paspaliari, Elif Seyda Tosun, Alieda van Essen-Zandbergen and René S. Hendriksen	Apr 2026	René S. Hendriksen

Background

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry is a core analytical technique for rapid and reliable microbial identification. In the MALDI Biotyper Sirius workflow, bacterial material from a fresh, pure culture is applied to a reusable MALDI target plate, following one of the preparation strategies described in the method SOP (e.g., direct transfer, direct transfer with formic acid, or extraction). The sample is overlaid with an α-cyano-4-hydroxycinnamic acid (HCCA) matrix, which absorbs the laser energy and facilitates the desorption and ionization of microbial proteins.

A critical safety requirement in the SOP is that all samples and reagents on the target plate must be completely dry before the plate is inserted into the instrument, as residual moisture can severely damage the detector and void the Bruker warranty. Proper drying also ensures optimal matrix crystallization, which is required for generation of high-quality spectra.

Once dry, the MALDI target plate is placed into the MALDI Biotyper Sirius with the carrier in the OUT position and the O-ring inspected to ensure a clean vacuum seal, as specified in the equipment instructions. Inside the instrument, the crystallized matrix–analyte mixture is irradiated by a UV laser, resulting in ionization of predominantly ribosomal proteins. These ions are accelerated through a vacuum tube, and their time of flight to the detector is measured. Because ions separate according to their mass-to-charge ratio (m/z), the output is an organism-specific spectral fingerprint.

Spectra are acquired in flex control and processed in MBT Compass, which matches the generated profile against curated reference libraries (BDAL, SR, and internal DTU databases) to produce an identification. Successful identification typically requires a protein profile corresponding to $\geq 5 \times 10^3$ CFU/mL. Through this combination of standardized sample preparation, controlled instrument operation, and database-driven spectrum interpretation, the MALDI Biotyper Sirius enables rapid, high-throughput taxonomic identification fully aligned with ISO 17025 requirements for validated methodologies.

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Materials

Equipment & Consumables

- MALDI Biotyper Sirius (MBT) instrument
    - Note that it is very important to keep the door closed all the time as the machine needs to maintain a constant temperature (temperature range 16-33°C and system performance is optimal at 18-25°C).
- Reusable MALDI target plates (e.g., MSP 96 target, Bruker)
- Wooden toothpicks or sterile inoculation loop
- Test material: Fresh pure cultures (must not have been refrigerated after incubation; required for valid protein profiling)
- Eppendorf tubes
- Nitrile gloves (EN ISO 374-1:2016 Type B or A, required for chemical handling)
- Kleenex lint-free tissues (e.g., Abena Ref. 6210)

Chemicals & Reagents

- HCCA – α-cyano-4-hydroxycinnamic acid matrix powder (Sigma-Aldrich / Bruker pre-portioned HCCA)
- ACN – Acetonitrile, HPLC-grade
- TFA – Trifluoroacetic acid
- 100% TFA (for preparing 80% TFA cleaning solution)
- 80% TFA in-house prepared solution (used for cleaning MALDI target plates
- FA – Formic acid (70%) for direct transfer + FA method or extraction protocols
- Ethanol 70% – For cleaning MALDI target plates
- Ethanol 100% (HPLC-grade) – Used during protein extraction protocols
- HPLC-grade water
- Milli-Q water (for rinsing plates after TFA cleaning)
- Bruker standard solvent (Sigma-Aldrich 900666)

Calibration reagents (when applicable)

- Bacterial Test Standard (BTS), Bruker (5 × 50 µL)
- HCCA Matrix pre-portioned tubes for calibration in case of in-house production (Bruker)

Procedure		Theory/comments
1. Standard procedures for bacterial identification using MALDI biotypes		
1.1. Direct transfer of biological material: Apply with a wooden toothpick a thin layer of colony material from fresh culture onto a target plate and transfer 1 µl HCCA Matrix to each spot. (Always change pipette tip for aspirating Matrix). After completely drying at room temperature, the target plate is ready to be placed in the MALDI-TOF instrument.		When analyzing biological material on MALDI - generally 3 different ways of treatment of the sample material can be used. The optimal method may depend on the species - if no valid result is obtained by direct transfer (1) of colony material, this may be remedied by method 2 or 3. For best results, preparation of all solutions, standard solvent, and the entire sample preparation process including drying steps must be performed at controlled room temperature, defined as 20-25°C. The Bruker MALDI-TOF mass spectrometer must be operated within the temperature range of 16 – 33°C. When setting up on a target plate, the sample material is typically transferred to each of three spots (triplicates) - see set-up scheme at the back. There are 8 x 12 = 96 spots on the target plate and samples are applied in sequence - i.e. a target plate is 'reused' until all spots have been applied, after which the target plate is cleaned as described below. Already used spots will be remembered by the MALDI Biotyper by scanning the target plate barcode before running (see under operation).
1.2. Direct transfer plus formic acid Using a toothpick or loop, smear biological material from a fresh pure culture as a thin film onto spots on the target plate. Avoid agar! Allow the material to dry. Transfer 1 µl 70% formic acid to each spot. Always use new tip. Allow to dry completely. Transfer 1 µl HCCA matrix to each spot. Always use new tip. Allow to dry completely. The plate is ready for analysis in MBT.		Use fume hood. A methodology preferably for mucoid bacteria
1.3. Protein extraction Transfer 300 µl HPLC water to an Eppendorf tube.		Use fume hood.

Procedure		Theory/comments
Add colony material to the water with an inoculation loop (1 µl). Mix until the mixture is homogeneous. Add 900 µl absolute HPLC-grade ethanol, vortex for 1 minute. Centrifuge for 3 min at approximately 14,000 rpm. Discard the supernatant. Repeat centrifugation and remove supernatant with pipette. Allow pellet to dry for a few minutes. Add 50 µl 70% formic acid, mix with pipette followed by vortexing. Incubate at room temperature for 1-3 min. Add 50 µl 100% acetonitrile, mix with pipette. Centrifuge 3 min at approx. 14,000 rpm. Place 1 µl supernatant on target plate. Allow to dry. Place 1 µl HCCA matrix on the target plate. Allow to dry completely. The plate is ready for analysis in MBT.		
2. Test run procedure		
2.1. In MBT Compass, position the cursor in "Enter target or run ID" field. Scan the Target plate barcode. The plate ID and date are displayed in the field. The laser automatically moves to the next available position on the target plate. If you have used a clean plate and your samples start in A1, press RESET TARGET. The laser and cursor will move to position A1.		Samples and reagents MUST BE COMPLETELY DRY before the target plate is inserted into the instrument Moisture in the instrument will destroy the detector and void the warranty from supplier The MALDI-TOF is always on. It is only switched off by a BRUKER technician during service or when a BRUKER service technician instructs by telephone. The device is operated using the associated computer. The scanner is located next to the MBT. This is used to scan the reusable MALDI target plates, before inserting them into the MBT. The device is operated using the associated computer and the MBT Compass program. The procedure for using the equipment is described in the

Procedure		Theory/comments
		manufacturer's manual MBT Compass user Manual [1] The FlexControl program runs in the background.
2.2. Press the button on the front of the instrument (or the load/eject icon in FlexControl), the carriage will eject. When inserting target plate into the instrument, the carrier slate must be in the OUT position, which is done by pressing the button on the front of the instrument. After inserting the target plate, before closing the lid, make sure that the O-ring is free of dust and other small elements, that could hinder the creation of the vacuum. (Figures 1 and 2). Close the lid and press the button again and the carriage retracts. When the "IN" box in the lower right corner of the Flex Control is green: • Continue in MBT Compass on the computer. • Click "speech bubble icon" and enter your initials and any other info you wish. • Enter sample numbers/ names in the lines in Compass. • Await green "READY" bottom right corner in Flex Control before continuing. • Press start acquisition (Bottom right). • At the end of the run, remove the plate: press the button - remove the plate. • Press the button so that the carriage retracts - check that the "IN" field in Autoflex is green.		In the lower right corner of FlexControl, it is indicated when the carriage is in the "out" position by the field illuminated green. The slide should always be in the "in" position, except when inserting or removing a target plate. This is to maintain proper vacuum in the laser chamber. New lines will appear when "Enter" is pressed. Note: This step might differ depending on the version of the instrument e.g. enter the run info in a different place and press a button in the top left to start the run.
2.3 Save results: • Click 'Archive run'. • Save data in the PC drive (D:) under 'Data' > 'Archive data' > personal folder (or other relevant folder). • If you do not have a personal folder or other relevant folder then Create one		Browse to save the data on e.g. a local drive. The file is saved as a zip file. Right click on the file, select 'extract all', click on 'extract'. Close the window. Now, there is a new type of file (check that there are two files with the same name), which is the one to be saved. Delete the zip file

Procedure		Theory/comments
• The data will be saved as a .zip file. Extract the contents. • After extracting delete the zip file and close the window. • Click 'Report run'. This generates a PDF file with your results. Save the PDF in the same personal folder as before. 2.4 Handing over to next user • Remove target plate. • Clean O-ring, close carrier slate and return carrier slate to IN position. • Note that you used the MBT in the log book as well as the run status • Any issues during run ? Also note the problem and actions taken • Leave Compass and Flex open. • Exit the run by clicking 'Home' in Compass. • Check if there are still unused spots on target plate • If not, then clean plates		(right click on it and select delete). Results may also be saved as a PDF. Note: This step might differ depending on the version of the instrument e.g. some download data as a csv-file which can be saved as a PDF.
2.5 Calibration and detector check Calibration and detector check must be performed every 14 days. Calibration and detector check are performed on the same BTS spot. **Calibration:** • Load a target plate with 1 spot BTS (1 µl BTS, let dry, 1 µl HCCA matrix, let dry) in the Maldi. • In Flex Control click in the videoscreen on the position of the BTS spot. The target plate will now shift so that the corresponding position becomes visible on the FlexControl video screen. • Click the 'Calibrate' button. The calibration will start. • When the calibration is complete, a pop-up screen will appear. • Register the standard deviation, max. peak error and the height of the highest peak in the log sheet.		Cell debris and matrix accumulate in the detector with use. To ensure good analysis the detector needs cleaning occasionally (source cleaning). Always make sure to clean the O-ring from dust, before closing the lid to the target plate carrier, by running a finger along it.

Procedure		Theory/comments
Detector check: • In Flex Control, click the tab 'Status' • Click the grey button "Detector Check". • Click the grey button "Start Check". • Tick the 3 boxes and click "OK". • The detector check will take about 5 minutes. • Register the detector check in the log sheet. 2.6. Self-cleaning of the detector • In Flex Control, click the tab 'Status' • Click the grey button 'Details' • At the top right, check 'source clean %' • If 'Source clean %' is above 80%, Source clean should be performed. • Source clean takes about 20 min. Check if there is time enough before the next reservation. • Register the cleaning in the Source clean log sheet (APPENDIX 4). Write your initials and date as well as the detected percentage before the cleaning.		
2.7 Reading the results **Category** / **Assessment** / **Symbol** 2.00 … 3.00 / ID on species level / (+++) 1.70 … 1.99 / ID on genus level / (+) 0.00 … 1.69 / No safe ID / (−)		If you are performing analysis other than the one described above, please leave the PC in 'normal mode' so that subsequent users do not have to spend time finding the correct settings
2.8 To clean the target plates: Clean the target plate by scrubbing intensive with a tissue and 70% ethanol, make sure also the corners are done well. Under running tap water, wipe biological material off the surface with a tissue. Scrub the plate again with a tissue and 70% ethanol and rinse with running tap water. Collect the tap water in the small container. Wipe the plates with the Kleenex. These Kleenex are chemical waste group B and must be disposed		The reusable target plates are used continuously until all the spots have been occupied. This means that when using the MBT you can use a plate that already has some samples in it. If there are not enough spots available for the tests you want to perform you must clean the plates before starting the test. If you fill up a reusable plate during your test you must clean it afterwards so that other colleagues have plates to use. If you clean one plate, clean all other plates

Procedure		Theory/comments
	of in the proper waste container inside the fume hood. Transport the container with the tap water to the fume hood. Transfer the water into the chemical waste container inside the fume hood. Put the container with the tap water inside the fume hood. Continue in the fume hood using nitrile gloves. Pipette 100 µl of 80% TFA onto the surface of plate. All the waste from this point onwards incl. liquids is chemical waste group B and must be disposed of in the proper waste container inside the fume hood. Spread the TFA with Kleenex and wipe with Kleenex, make sure also the corners are done well. Rinse with Milli-Q water and wipe dry with Kleenex. Collect that waste (water and Kleenex) in the waste container inside the fume hood. Allow the plate to dry for minimum 15 min. at room temperature before use. The plate must be completely dry before use or storage.	that are used or partly used to ensure that other colleagues have material available.
2.9	Testing the reusable MALDI target plates Clean the MALDI target plate as described above. Deposit 1 µL BTS solution onto MALDI target plate positions that are affected by scratches. Carefully inspect the MALDI target plate and make sure that spots are well-separated from each other and that none of the spots has bled into a neighbouring position. If all spots are well-separated from each other, proceed to step (below). Air-dry the BTS spots at room temperature and overlay each position with 1 µL HCCA matrix solution. If HCCA matrix solution is not added to samples within 30 minutes after they have dried, these positions cannot be tested. Dry the spots at room temperature. Carefully inspect the MALDI target plate and make sure that spots are well-separated from each other and that none of the spots has bled into a neighbouring position.	Over time, MALDI target plates may develop deep scratches on their surface. Use the following procedure to test the suitability of the reusable MALDI target plates if there is any doubt regarding their quality.

Procedure		Theory/comments
Before proceeding, confirm that the MALDI target plate is completely dry. If all spots are well-separated from each other, load the MALDI target plate into the MBT and measure the BTS QC positions. High-confidence Escherichia coli identifications with a score ≥ 2.0 should be obtained. If the expected high-confidence Escherichia coli identifications are not obtained, repeat steps (1)–(6) until high-confidence identifications are obtained. If a spot has bled into a neighbouring position in points 3 or 6, the MALDI target plate is unsuitable and should be disposed of.		Store cleaned MALDI target plates in a dry place at room temperature in the supplied container. Avoid exposing cleaned MALDI target plates to potential sources of contamination (for example, dust) or corrosive atmospheres. After cleaning, do not touch the upper surface of the MALDI target plate. Do not place any adhesive labels on the MALDI target plate. Do not drop or scratch the MALDI target plate.

Figure 1

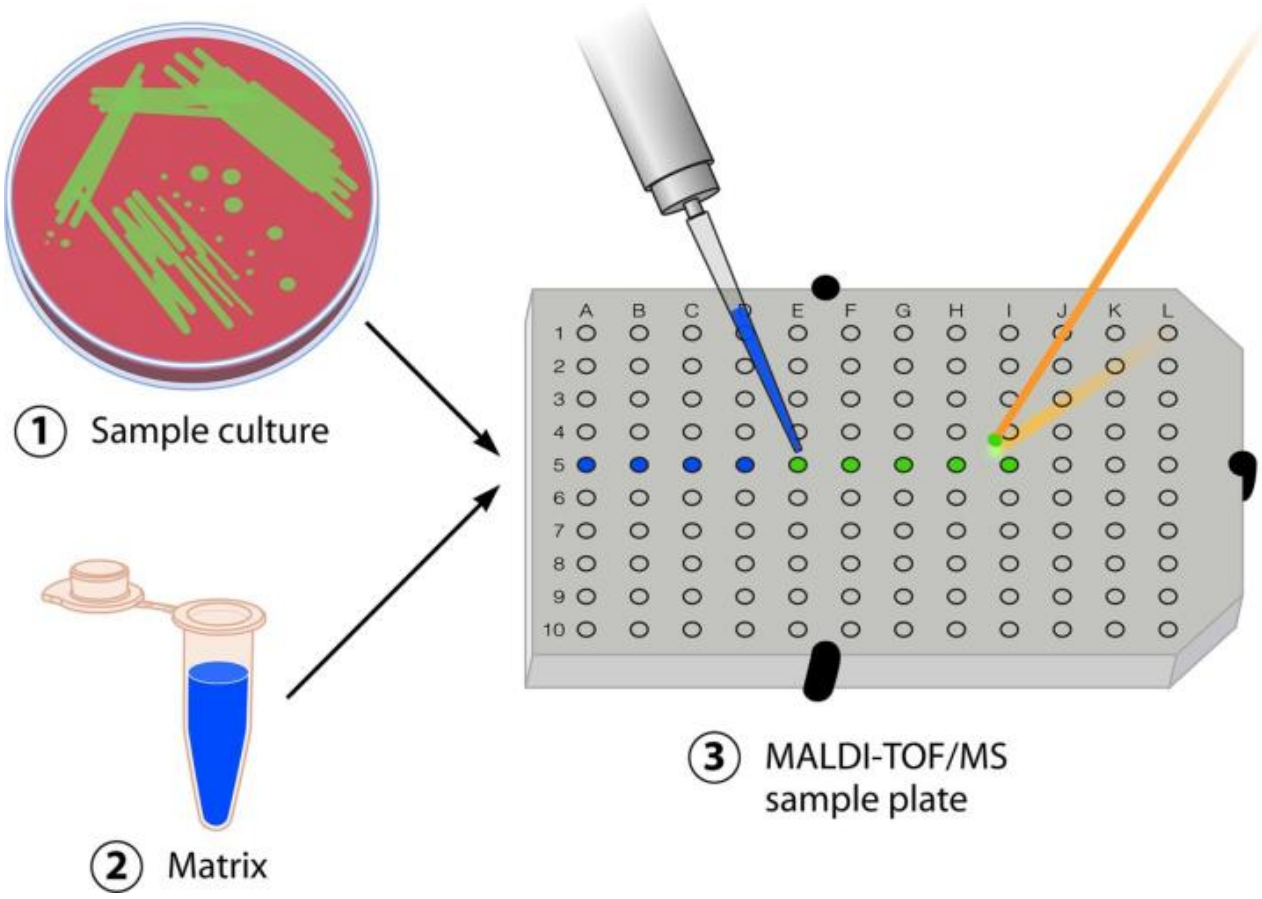
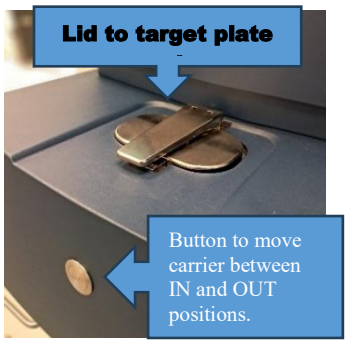


Figure 1. Simple flow chart of the MALDI-TOF procedure

Figure 2



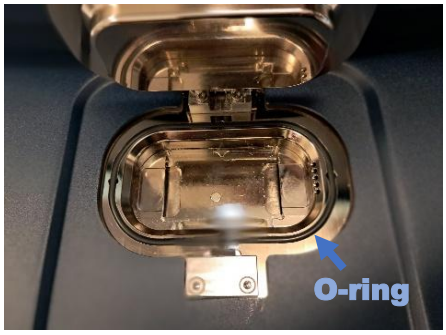


Figure 2 (left). Lid to target plate carrier and button to move the carrier between IN and OUT positions.

Figure 2 (right). Target plate carrier, with open lid, showing O-ring.

Figure 2. Key parts of the MALDI-TOF instrument

References

1. Bruker MBT Compass User Manual Ref 1843241 Revision E (April 2019)
2. Bruker flexControl 3.4 User Manual Revision 1 (November 2011)

APPENDIX 1

Chemicals and preparation of reagents:

The buffered peptone water (BPW), MacConkey agar media and reagents are available from several companies. The composition of the dehydrated media given below is an example and may vary slightly among the different manufacturers. Of note, the media should be prepared according to the manufacturer's instructions, if they differ from the description given here.

- Only HPLC-grade chemicals specifically purchased for MALDI-TOF analyses may be used.
- The chemicals should be kept in a ventilated chemical cabinet and stored at room temperature.

HCCA matrix must be added to each analytical sample - and is prepared by adding 'standard solvent' (acetonitrile 50%, water 47,5% and trifluoroacetic acid 2,5%)to HCCA matrix powder. HCCA matrix has a shelf life of 14 days (at room temperature - protected from light).

When preparing a new HCCA matrix, a fresh solution of 'standard solvent' must be used.

Preparation of 'standard solvent' - ACN-TFA solution, 1 ml:

- 475 µl water (HPLC grade)
- 500 µl 100% Acetonitrile (ACN)
- 25 µl Triflouroacetic acid (TFA)

1. Transfer the chemicals to an Eppendorf tube in the same order as above.
2. Mix thoroughly on vortexer.
    a. All work with 'standard solvent' - ACN-TFA solution must be carried out in a fume hood.
    b. Pay attention to the hazard labels - use of protective equipment and disposal of waste!

Or use Bruker standard solvent (Sigma-Aldrich)

Preparation of HCCA matrix (for analysis of samples):

1. Add 250 µl standard solvent to a tube with HCCA matrix powder .
2. Mix thoroughly on vortexer. There will be undissolved yellow crystals at the bottom of the tube.
    a. Shelf life 14 days, note date of making on lid.
    b. Store tube at room temperature.
    c. Vortex before each use.

Production of 80% TFA: (used for cleaning target plates)

1. 2 ml HPLC-grade water/sterile MQ water

2. Add 8 ml 100% TFA, vortex 1 minute
    a. Prepare in small brown glass bottle in fumehood, store in chemical cabinet.
    b. Add correct safety label.

APPENDIX 2

Hazard and Precautionary Statements

- Pay attention to the hazard labels of the reagents/chemicals used
- Wear gloves when handling chemicals (but not when opening drawers/cabinets etc.!)
- Mixing of reagents should be done in the fume hood.
- Dispose of waste in the correct container, check the labelling.

ACN = Acetonitril

Hazard Statements (H-phrases)

H225: Highly flammable liquid and vapour.

H302 + H312 + H332: Harmful if swallowed, in contact with skin, or if inhaled.

H319: Causes serious eye irritation.

Precautionary Statements (P-phrases)

P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

P261: Avoid breathing vapours.

P280: Wear protective gloves/eye protection.

TFA = Triflouracetic acid

Hazard Statements (H-phrases)

H314: Causes severe skin burns and eye damage.

H332: Harmful if inhaled.

H412: Harmful to aquatic life with long lasting effects.

EUH071: Corrosive to the respiratory tract.

Precautionary Statements (P-phrases)

P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P314: Get medical advice/attention if you feel unwell.

P273: Avoid release to the environment.

Formic acid (FA)

Hazard Statements (H-phrases)

H226: Flammable liquid and vapour.

H331: Toxic if inhaled.

H302: Harmful if swallowed.

H314: Causes severe skin burns and eye damage.

EUH071: Corrosive to the respiratory tract.

Precautionary Statements (P-phrases)

P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

P260: Do not breathe vapours or aerosol mist.

P280: Wear protective gloves/eye protection/face protection.

P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P304 + P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing.

P370 + P378: In case of fire: Use dry powder or dry sand to extinguish.

Ethanol, 100% og 70%

Hazard Statements (H-phrases)

H225: Highly flammable liquid and vapour.

H319: Causes serious eye irritation.

Precautionary Statements (P-phrases)

P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P280: Wear protective gloves/eye protection.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313: If eye irritation persists: Get medical advice/attention.