**National Department of Health**

# Title: Bruker MALDI-ToF Biotyper System

ID: G_90_SOP_14_A

| | |
|---|---|
| **Developed by:** | **T Ikanofi, T Oakley** |
| **Reviewed by:** | **W Jackson, J Ferguson** |
| **Authorized by:** | **W Porau** |
| **Issue date:** | **12/10/22** |
| **Review period:** | **2 years** |

**Changes to the last authorized version:**

| Version | Date issued | Changes |
|---|---|---|
| A | 12/10/22 | New document |

**Certification of printed copy:**

| | |
|---|---|
| Version | |
| Authorised by (name) | |
| Signed | |
| Date | |

*NB. Printed copies of this document for local laboratory use require endorsement on the front page and recording on the Printed Controlled Document Logsheet G_10_WS_5.*

## 1. Purpose & Scope

This document outlines the procedure for identification of bacterial and fungal isolates from culture media using the Bruker MALDI-ToF[1] Biotyper System platform.

## 2. Principle & Clinical application

[1] Matrix-assisted laser desorption/ionization time of flight mass spectrometer: https://en.wikipedia.org/wiki/Matrix-assisted_laser_desorption/ionization

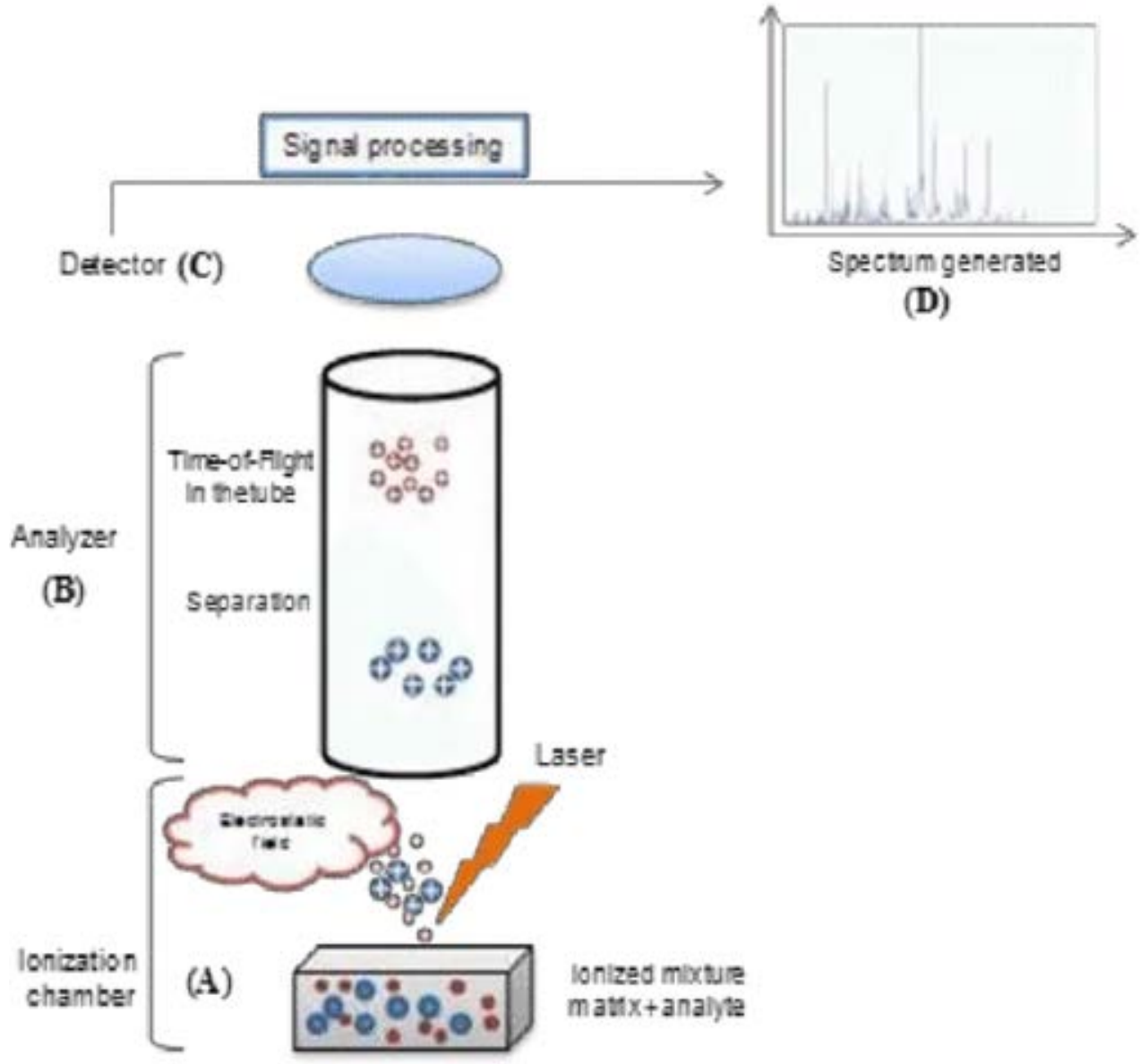
*NB. Printed copies of this document for local laboratory use require endorsement on the front page and recording on the Printed Controlled Logsheet G_10_WS_5.*

**Principal of MALDI-ToF MS**

Charged target plate in ionization chamber desorbs microbial and matrix molecules by ultraviolet laser creating an ionized state. Ionized microbial molecules are funneled through a positively charged electrostatic field into the time of flight mass analyzer toward an ion detector. Ions separated by molecular weight, collide and accelerate hitting the detector over time. This generates characteristic spectral fingerprint that is compared with mass spectra of well characterized organisms available in the reference library database to identify the isolate.

**Clinical application**

MALDI-ToF system is a rapid and accurate method used for diagnostic purposes to identify pathogenic bacteria and yeast associated with clinical disease.




*(Image source: Cheikh Ibrahima Lo)*

## 3. Responsibilities

| Role | Person responsible |
|---|---|
| Oversight of all staff who are authorized to use the MALDI<br>Main onsite trainer<br>Preparation of the BTS standard (reconstitution and aliquoting)<br>Supervise maintenance and machine<br>Evaluate QC and BTS performance | Authorised Superuser; initially Ms T Ikanofi |
| Daily, weekly and monthly maintenance<br>Run appropriate QC controls with each daily run<br>Weekly run of one aliquot of BTS (Monday) | Specified laboratory scientists who have been trained, competency asssessed and authorized locally by one for the local Superusers above |

## 4. Specimen

Single microbial (bacterial or fungi) colony from an overnight growth of a pure culture
Slow growing organisms may need several days to grow before testing

## 5. Equipment & Materials

- MALDI ToF MS Bruker Sirius Biotyper
- Bruker MALDI target plate
- Bruker Standard Solution (Weekly, aliquot 1 mL Brucker Standard Solution to working tube)
- Prepared Bacterial Test Standard (BTS)
- Prepared Bruker HCCA matrix solution (A-Cyano-4-Hydroxycinnamic Acid)
- 70% Formic Acid
- Wooden applicator sticks
- 0.5–10 µL micropipette
- ATCC control bacterial strains and wild type *Candida albicans*

## 6. Safety

All institutional safety procedures must be followed. Formic acid is toxic and corrosive while HCCA is skin and eye irritant. See also Appendix.

For other safety aspects, please review this document G_10_Info_3 Laboratory Biosafety.

## 7. Procedure

### 7.1. Reconstitution and preparation of BTS test aliquots

- Remove the original BTS source container from the -20°C freezer (yellow lid)
- Add 50 µL of Bruker Standard Solvent (BSS) and dissolve pellet by pipetting suspension **slowly** up and down at least 20 times.

  Try not to produce any bubbles or foam when mixing.
- Incubate at room temperature for 5 minutes
- Mix suspension again by **slowly** pipetting up and down for another 20 times.

  Try not to produce any bubbles or foam when mixing.
- Centrifuge at 13,000rpm for 2 minutes
- Label 6 sterile Eppendorf tubes with 'BTS' and date of production using a permanent marker
- Aliquot 7µL into each of the 6 tubes
- After this procedure there should be 6 aliquots and the BTS original tube (50µL).
- Store at -20°C in a rack.

*Note: BTS aliquots can be kept for up to 5 months when stored at -20°C.*

### 7.2. HTCC Matrix preparation (weekly on Mondays)

- Remove one HCCA Matrix vial from fridge and allow to warm to room temperature.
- Add 250 µL of Bruker Standard Solvent (BSS)
- Vortex until completely mixed and no dry solution can be seen on the bottom of the tube
- Initial and date the prepared matrix tube.
- Store at room temperature in a cool dark box
- Use within 7 days

### 7.3. Formic Acid preparation

- Label a screw top tube with 70% Formic Acid and date of preparation
- Add 700µl >=95% Formic Acid
- Add 300µl distilled water
- Mix by inversion
- Store in dry cool place (room temperature)

*Note: this made-up solution of 70% Formic acid can be kept for up to 1 month.*

### 7.4. BTS Quality Control (run weekly on Mondays)

- Retrieve one aliquot of BTS from -20°C freezer
- Centrifuge for 15 seconds (quick spin/pulse)
- Mix gently by pipetting up and down 10 times
- Pipette 1 µL of BTS onto the assigned BTS QC position
- Ensure the BTS aliquot is promptly returned to the freezer
- Completely dry spots on target plate at room temperature
- Immediately overlay each position with 1 µL HCCA matrix solution
- Completely dry spots on target plate at room temperature
- If spots are not dry within 5 minutes contact a supervisor

### 7.5. Bacterial identification

- Record isolate details (lab number and patient ID) on the MALDI ToF daily worksheet (G_90_WS_13)
- Pick a discrete, distinct colony from fresh culture plate and smear (thin film) onto a spot on the MALDI target plate using a wooden applicator stick

*Organisms that have been stored at 4˚C or lower have a negative impact on spectra quality and reproducibility .*

- Pick a colony of the assigned QC organisms for the day and smear onto the spot on the MALDI target plate designated QC.
- Pipette 1 µL of FA to the assigned negative control position. Completely dry FA spot on target plate at room temperature
- Immediately overlay each sample position with 1µL HCCA matrix solution[2]

*Use a new pipette tip for each sample position to avoid cross-contamination. Note: If HCCA matrix solution is not added to samples within 30 min after they have dried, these positions cannot be tested.*

- Completely dry spots on target plate at room temperature
- If spots are not dry within 5 minutes contact a supervisor
- Place the MALDI target plate into the plating chamber of the mass spectrometer loading port
- Trace index finger around vacuum seal of loading port to secure the seal

### 7.6. Fungal identification

- Record isolate details (lab number and patient ID) on the MALDI ToF daily worksheet
- Pipette 1µL Formic Acid (70%) on to the MALDI target plate to each spot that will be used for sample analysis

[2] The organic solvent in the matrix solution extracts proteins (mainly ribosomal proteins which are present in high concentration) from the microorganisms.

- Emulsify an isolated colony from culture plate into the Formic Acid using a wooden applicator stick. *Note: use a small amount of picked colony material to maintain a thin film*
- Apply positive and negative controls as per section 7.5 above
- Immediately overlay all sample and control positions with 1µL of HCCA matrix solution

*Use a new pipette tip for each sample position to avoid cross-contamination. Note: If HCCA matrix solution is not added to samples within 30 min after they have dried, these positions cannot be tested.*

- Completely dry spots on target plate at room temperature
- If spots are not dry within 5 minutes contact a supervisor
- Once the target plate is dry, the plate must be run within 24 hours
- Load the MALDI target plate into the mass spectrometer

### 7.7. Target Registration in Bruker MALDI Biotyper

- Ensure MTB Compass and FlexControl are open
- Click 'Home'
- Scan target barcode and click '+ Run'
- If a clean or new target plate click 'Reset Target'
- Ensure your target starting position mirrors the first inoculated spot
- Scan/add accession number for each target position. Press enter and it will automatically bring up the next spot
- Enter in daily QC and negative control details
- Add description as necessary for multiple isolates from one accession number. Ensure the target inoculated with BTS is ticked and has 'BTS' in the ID field column
- In flexControl go to:-
    - 'Status'
    - 'Details'
    - 'Vacuum'

*Check the 'Source Rough' pressure under Gauges is* ***less than 3.0e+00 mbar*** *and that Processor Subsystem, High Voltage and Vacuum are 'Ready.' At this time the analyser LED should show a steady white light.*

- Click 'Start Acquisition'
- After the run is complete:
    - Review results
    - Print a copy and store with the MALDI-ToF worksheet
    - Leave the target plate in the machine (in the 'in' position) until the next run
    - Leave the plastic holding container for the target plate on the machine to indicate a target plate is still inside.

### 7.8. Interpretation of instrument Status using the LED on the front of the instrument

**Table 1: System status information (Status LED strip)**

| Color and State | Instrument Status | Description |
|---|---|---|
| Steady white | NORMAL or STARTUP | The system is ready for loading/unloading a MALDI target plate or for starting measurements. - The instrument is starting up. |
| Flashing white slow | DOCKING | MALDI target plate loading procedure is in progress. |
| Flashing white fast | AUTOMODE | Instrument is performing a measurement. The **IN/OUT** button is not available. |
| Steady red | ERROR | Malfunction that requires an intervention of user or service technician. |

## 8. Results Recording

**Meaning of Score Values**

| Range | Description | Symbols | Color |
|---|---|---|---|
| 2.300 ... 3.000 | highly probable species identification | (+++) | green |
| 2.000 ... 2.299 | secure genus identification, probable species identification | (++) | green |
| 1.700 ... 1.999 | probable genus identification | (+) | yellow |
| 0.000 ... 1.699 | not reliable identification | (-) | red |

**Meaning of Consistency Categories (A - C)**

| Category | Description |
|---|---|
| A | **Species Consistency:** The best match was classified as 'green' (see above). Further 'green' matches are of the same species as the first one. Further 'yellow' matches are at least of the same genus as the first one. |
| B | **Genus Consistency:** The best match was classified as 'green' or 'yellow' (see above). Further 'green' or 'yellow' matches have at least the same genus as the first one. The conditions of species consistency are not fulfilled. |
| C | **No Consistency:** Neither species nor genus consistency (Please check for synonyms of names or mircobial mixture). |

## 9. Interpretation

Using direct colony transfer and the manufacturer's recommended criteria, cutoff of ≥1.7 and ≥2.0 is used for the identification of microbial genus and species respectively.

Also, check that the identification reported is consistent with the culture results and patient information.

Some identifications require further testing.

## 10. Quality Control

For each run include a pure culture of positive control organisms (bacteria or fungi) and a negative control. Weekly, include BTS.

Maintain an operating range of 15-85% humidity and 10-30˚C temperature .

## 11. Related documents – for access, visit https://path-png.org/microbiology-sops-fleming-fund/

| | |
|---|---|
| Target preparation and Registration (Job Aid) | G_90_J_2 |
| Cleaning and reagent guide | G_90_J_9 |
| MALDI-ToF Harsh Cleaning and Reagent Preparation Guide | G_90_J_21 |
| MALDI-ToF Daily maintenance and QC | G_90_WS_12 |
| Daily worksheet | G_90_WS_13 |
| MALDI-ToF Competency assessment) | G_90_COMP_12 |

## 12. References


- East Timor, Fleming Fund Country Grant documents
- Fraser, M., et al. (2016); "Rapid identification of 6328 isolates of pathogenic yeasts using MALDI-ToF MS and a simplified, rapid extraction procedure that is compatible with the Bruker Biotyper platform and database." Medical Mycology 54(1): 80-88.
- http://www.pharmatips.in/Articles/Pharmaceutical-Equipment/Standard-Operating-Procedure-Of-BRUKER-AUTOFLEX-MALDI-TOF-Mass-Spectrometer.aspx

# Appendix: Competency and training notes

## 1. Process for test set up and result review competency assessment (refer to G_90_COMP_12 MALDI-TOF Competency assessment)

| **Requirements** |
|---|
| a) Read the current MALDI-TOF SOP version and related documents |
| b) Safety aspects discussed by designated trainer as below |
| c) Observe test set up performed by trainer |
| d) Trainer observes trainee set up test (twice) |
| e) Achieve correct species-level identifications for test panel of assigned isolates |
| f) Competency assessment questionnaire completed |
| **Trainer and trainee sign off on competency level achieved on G_90_COMP_12.** |

## 2. Training notes

**Safety aspects**

**1/ Handling microbial cultures**

Slides for Neisseria cultures and bacterial cultures of unknown identity must be prepared in the BSC

**2/ Matrix and Formic Acid**

May be harmful in contact with skin, inhaled, or swallowed. Avoid contact with clothing.

**3/ Equipment**

Do not remove any hardware from platform –risk of lethal voltages and exposure to radiation.

- Keep liquids and flammable vapours away from the MALDI-TOF
- Do not stretch, twist or coil the power cable
- Do not restrict or block the airflow at the back of the MALDI-TOF - the instrument may overheat resulting in a fire

**Avoiding sample mix-ups**

This procedure involves working with many cultures simultaneously.

To avoid sample mix-ups:

- Work with one plate at a time
- Enter the number to the slide position on working sheet, and then apply culture to the slide
- If working in a BSC, record the slide coordinates on the plate

**Other critical aspects**

Do not load wet slides into the MALDI-TOF

Wear gloves when handling plates, remove gloves when loading target plates