Public Health England

NHS

UK Standards for Microbiology Investigations

Matrix-assisted laser desorption/ionisation - time of flight mass spectrometry (MALDI-TOF MS) test procedure

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Amendment table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment no/date.	1/27.11.19
Issue no. discarded.	1
Insert issue no.	1.1
Section(s) involved	**Amendment**
4. procedures and results	Direct plate extraction has been updated and acetronile has been removed from protocol.
Appendix 1	Algorithm updated and acetronile has been removed from direct extraction protocol.

Amendment no/date.	-/10.10.16
Issue no. discarded.	-
Insert issue no.	1
Section(s) involved	**Amendment**

UK SMI[#]: scope and purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratorieshttp://www.hpa-standardmethods.org.uk/. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the SMI working groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The SMI working groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs is subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of SMIs, PHE and the partner organisations, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made by an end user to an SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the date of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Scope of document

This SMI describes the test procedure for MALDI-TOF MS and its use in the examination of cultures obtained from clinical specimens as mentioned in the UK SMI Identification (ID) documents. This document includes the mechanism as well as the limitations of the technique in its use in diagnostic microbiology laboratories.

For information on evaluation and validation of this method for use in the laboratory, refer to Q 1 - Evaluations, validations and verifications of diagnostic tests.

Refer to specific ID documents for more information on the technical information/limitations of using this test procedure.

This SMI should be used in conjunction with other SMIs.

Introduction

Matrix-assisted laser desorption/ionisation - time of flight mass spectrometry (MALDI-TOF MS)

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is a soft ionisation technique used in mass spectrometry, allowing the analysis of biomolecules (such as DNA, ribosomal proteins, peptide and sugars) and large organic molecules (such as polymers, dendrimers and other macromolecules), which tend to be fragile and fragment when ionised by more conventional ionisation methods. The ionisation is triggered by a laser beam. It is a rapid and highly reliable analytical tool for the characterisation of a diverse collection of microbes encountered in the clinical laboratory.

This technique can be used to analyse the protein composition of a microbial cell, and has emerged as a new technology for species identification[1]. It has been shown to be a powerful technique because of its reproducibility, speed and sensitivity of analysis. The advantage of MALDI-TOF MS as compared with other identification methods is that the results of the analysis are available within minutes to a few hours rather than days. The speed and the simplicity of sample preparation and result acquisition associated with minimal consumable costs make this method well suited for routine and high throughput use[2].

Ultimately, MALDI-based identification systems may prove the most cost-effective means of identification dependent only on how comprehensive the databases are and the through-put of samples to warrant the initial substantial capital outlay[3]. A drawback with the currently available commercial platforms is that a number of well-established commercial manufacturers use their own algorithms, databases, software, and interpretive criteria for microbial identification, thereby making the new numerical output between these different commercial systems not directly comparable[4,5]. Users should be warned that some MALDI-TOF MS commercial software platforms might not be able to identify Hazard Group 3 pathogens unless the user applies to the commercial company for the full database, which will not normally be supplied due to bioterrorism concerns[4]. This could also potentially lead to additional testing of these isolates for further identification, thereby increasing the potential for exposure to such isolates to laboratory staff.

Given changing nomenclature and ongoing description of new species and emerging microorganisms, regular and ongoing updates to databases are imperative to ensure clinical utility. Databases should include entries representing major phylogenetic lineages within each species. It is possible for users to add their own mass spectral entries to enhance existing databases or create their own database by including locally important strains or strains not well represented in commercial libraries subject to validation[4].

It should be noted that antimicrobial susceptibility is not directly determined by this method as the species-specific proteins in the MALDI-TOF MS spectra are largely unaltered by antimicrobial susceptibility status[4]. An example is the direct discrimination between strains, such as Meticillin resistant *S. aureus* and Meticillin sensitive *S. aureus* strains which is a great challenge for MALDI-TOF MS applications in diagnostic laboratories[6]. There is currently no available universal platform for the rapid determination of antimicrobial resistance covering an extended spectrum of bacterial genera that can be implemented into the workflow of the clinical laboratory. However MALDI-TOF MS does offer the potential to detect carbapenemase production, but, further improvements and validation are required before antibiotic susceptibility testing becomes routine practice[5-12].

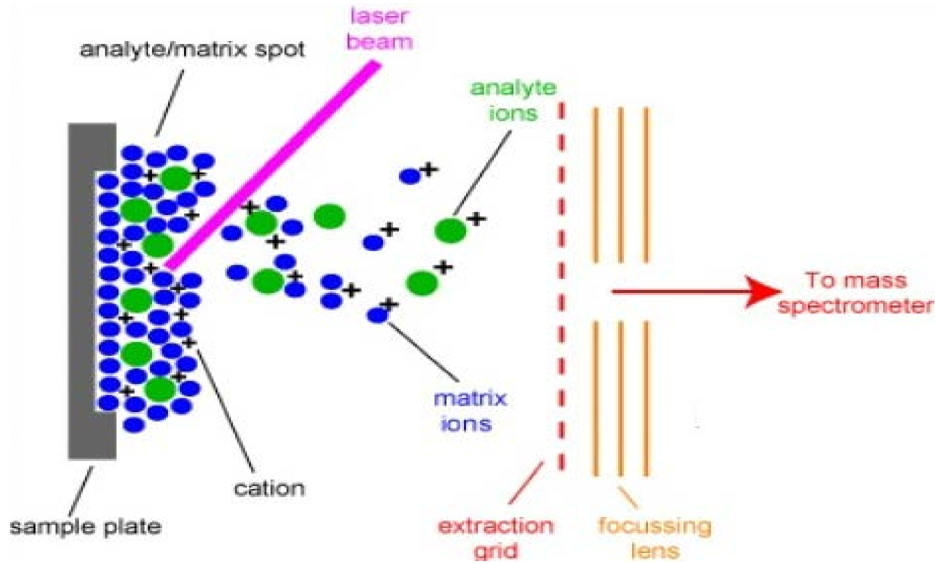
Whilst this method has been used successfully in the identification of bacteria and fungi, it has also been very useful for direct testing of clinical specimens such as urine, cerebrospinal fluid and blood which has resulted in significant improvements to patient care and reduced turnaround time to result[5,13]. Although, there are commercial validated kits for the direct extraction of these specimens (eg positive blood culture broths), further studies in specimen processing of blood culture broths and urine as well as improvements in the databases will be required prior to implementation. Clinical laboratories will also be faced with the challenge of selecting between MALDI-TOF MS methods and emerging molecular methods to identify bacteria from broth or directly from specimens[5,13].

Other strengths of MALDI-TOF MS

MALDI-TOF MS has other strengths listed below:

- it requires only a single colony overlaid with a matrix to perform the test in most instances (but not for yeasts or mucoid colonies)
- exposure risk is very low because samples are often inactivated by extraction before use
- very adaptable – open system, and easily expandable by the users
- requires minimal consumables
- useful in identification of bacteria that are difficult to culture such as Mycobacteria, *Bartonella* species, *Legionella* species, etc[14]
- useful in the identification of clinically relevant anaerobes such as *Bacteroides*, *Prevotella* and *Actinomyces* species as well as for the identification of Gram positive aerobes[15,16]
- useful in the identification of clinically relevant yeasts such as *Candida* species[17]
- specialist technical skills are not required

Principle of MALDI-TOF MS



Desorption ⇨Ionisation⇨Acceleration⇨Separation⇨Detection

(*Courtesy of Paul Gates 2014*)[18]

The basic procedure of MALDI-TOF MS is as stated:

- the target plate is placed into the ionisation chamber of the mass spectrometer. Spots to be analysed are shot by an ultraviolet N_2 laser desorbing microbial and matrix molecules from the target plate. The majority of energy is absorbed by the matrix, converting it to an ionised state

- through random collision in the gas phase, charge is transferred from matrix to microbial molecules

- the cloud of ionised molecules is funnelled through a positively charged electrostatic field into the time of flight mass analyser, a tube under vacuum

- the ions travel toward an ion detector with small analytes traveling fastest, followed by progressively larger analytes

- as ions emerge from the mass analyser, they collide with an ion detector generating a mass spectrum representing the number of ions hitting the detector over time. Although separation is by mass to charge ratio, because the charge is typically single for the described application, separation is effectively by molecular weight

Technical information/limitations

This is a compilation of some of the technical limitations/information that could be experienced by users when using the different platforms available. Not all will apply to each platform.

Presence of bacterial endospores

A limitation of this technique is the spectral interference due to the presence of spores in some organism species, for example *Clostridium* species. Younger cultures are used to minimise this interference[5,19].

Differentiation between organisms

Identification using MALDI-TOF MS relies on comparison of the spectra of the specimen/isolate with those of reference databases[1]. However the limitation of this technique is the inability of the mass spectrometry spectra to differentiate similar or closely related organisms such as *Escherichia coli* and *Shigella* species, some viridans streptococci and pneumococci, members of the *Candida albicans* complex etc.

MALDI-TOF MS can be unreliable in differentiating between pathogenic *Neisseria meningitidis* and non-pathogenic/opportunistic species, which has resulted in cases of *N.cinerea* and *N. polysaccharea* misidentified as *N. meningitidis* and vice versa which could have serious health, legal and social consequences[20-22]. Therefore in sensitive or critical situations, confirmation of *Neisseria* species identification should be confirmed with validated phenotypic or molecular methods.

Other organisms that are difficult to differentiate down to species level using MALDI-TOF MS include Mycobacteria, *Burkholderia* species, *Acinetobacter* species, Corynebacteria and β-haemolytic streptococci. This is due to their high degree of genetic similarity[23,24].

Existing taxonomical databases

MALDI-TOF MS databases can be improved by enhancing existing databases or by laboratories creating their own database by including locally important strains or strains not well represented or misrepresented in commercial libraries[4]. However, this requires a confident identification of the strains. Some examples are the misidentification of *Propionibacterium acnes* as *Eubacterium brachy*, misidentification between viridans streptococci and pneumococci, misidentification of *Pantoea agglomerans* as *Enterobacter* species, *Pandoraea pulmonicola* misidentified as *Sphingobacterium spiritivorum*[25-28].

Another problem commonly found in the routine identification by MALDI-TOF MS is error due to incorrect reference spectra in the database, although this is not that common with all systems.

Difficulty in lysing cell wall structures

Some organisms possess capsules which prevent efficient lysis of cells and results in a weak extraction yield and hence poor spectral quality. This may lead to problems with identification. Examples of such difficulties include differentiation between *Streptococcus pneumoniae* and *Streptococcus mitis* as well as differentiation between

most strains of *Haemophilus influenzae* and *Klebsiella pneumoniae*[25]. The key to overcoming some of these limitations lies in the quantity of inoculum and extraction method used[25].

Users are encouraged to test all isolates in duplicate because invariably, of the two spots, one has a better inoculum and gives a better log score (probability of correct identification)[29]. This maximises the chances of having a reportable result without further identification attempts and might be particularly valuable with mucoid colonies[30]. If testing in duplicate is used, the user needs to have a “reconciliation strategy” which must be explicit in local SOPs[23,24,31].

Culture media

Failure to identify some organisms may occur due to growth media used. The components of some media such as colistin-nalidixic acid agar or liquid media may result in potential interference[5,25].

Misidentifications

Examples that could lead to failure in identification include tiny or mucoid colonies, testing impure colonies, smearing between spots, failure to clean target plates and inoculating colonies in the wrong target plate locations[4,5].

Misplacing of the inoculum in the wrong spot can be minimised by duplicate testing (with reconciliation of discrepant results) and by re-testing results which are not congruent with the colonial morphology or the clinical data.

Identification of antimicrobial resistance

Antimicrobial susceptibility is not directly determined by this method as the species-specific proteins in the MALDI-TOF MS spectra are largely unaltered by antimicrobial susceptibility status[4]. There is currently no available universal platform for the rapid determination of antimicrobial resistance covering an extended spectrum of bacterial genera that can be implemented into the workflow of the clinical laboratory. Although MALDI-TOF MS is becoming increasingly available to diagnostic laboratories and it offers the potential to detect carbapenemase production, further improvements and validation are required before antibiotic susceptibility testing becomes routine practice[5-12].

Extraction methods

There are several extraction methods used in MALDI-TOF MS for the pre-treatment of clinical specimens/ isolates[5].

There is no single best recommended extraction method. Users should ensure that they use an appropriate extraction method as recommended by the manufacturer so as to get accurate identification results as well as to demonstrate that protein profiles remain consistent with database fingerprints. For example, yeasts require a protein extraction procedure to be correctly identified[25]. Filamentous fungi still lack standardised extraction protocols and commercially available databases lack the full scope required for fungal identification. However, MALDI-TOF MS is the preferred method of identification for filamentous fungi compared to morphology because of its robustness and accuracy. A continuous extension of its fungal database library is necessary to improve its reliability further[32,33].

Anaerobes can be identified using either chemical extraction or by using direct-smear methods. It has also been argued that in some cases, protein extraction may be detrimental to optimal spectral generation for anaerobes[34].

A summary of the examples for MALDI-TOF MS sample preparations for use with different classes of organisms can be seen in appendix 2[5].

Quality controls

Users should ensure the extraction procedures are conducted properly by trained competent staff. It should also be noted that all reagents used are in date and stored correctly.

The performance of the extraction step and of the MALDI-TOF mass spectrometer may be checked by testing a few selected bacterial strains routinely, for which spectra are available in the database. If any changes are to be done to any of the steps in the MALDI-TOF MS such as change in reagents, specific changes in the extraction protocols, this should be validated by the laboratory in question before it is used routinely. However, where modified extraction methods have been used, users should ensure that this is compatible with the existing database and where it is not; a new database may be created and used with the modified protocol after validation.

Appropriate servicing and maintenance of the MALDI-TOF MS equipment is also essential in order to obtain accurate results. This should be done more frequently if equipment is heavily used or located in a dusty or crowded area[25].

Results and interpretation reports

Error may also occur by wrong result entry into laboratory information systems and errors in the reference spectra due to incorrect reference spectra in the database[4].

Results should be interpreted with care and caution. This should be interpreted using the recommendations provided by the manufacturer of the platform used. If the result identification is still in doubt, the whole process should be repeated again.

Ideally the MALDI-TOF MS would be interfaced with the laboratory information management systems (LIMS) so that the microbiologist could see how many identification attempts have been made with or without extraction and the log scores that define the probability of an accurate identification. When there is no interface, the details of the method used, the number of the attempts and the log scores should be entered manually at least for the potentially “problematic” identifications in accordance with a local protocol. No identification system achieves 100% accuracy and the MALDI-TOF MS technique has a number of known weaknesses: the medical microbiologists should reconcile the laboratory identification with the clinical presentation and having information about the likely accuracy of the identification can help to decide when identification by alternative methods should be sought.

1 Safety considerations[35-51]

All work likely to generate aerosols must be performed in a microbiological safety cabinet. However, sometimes the nature of the work may dictate that full Containment Level 3 conditions should be used, for example, for the testing of *Brucella* species, *Bacillus anthracis*, *Mycobacterium* species, etc in order to comply with COSHH 2004 Schedule 3 (4e). Suspected CL3 organisms should be handled in CL3 laboratories by trained staff. Extra care should be taken when transporting a target plate with a suspected category 3 organism to the MALDI-TOF MS analyser in a sealed box. Any target plate with a proven category 3 organism on it is cleaned immediately and not used for other project runs.

Matrices are low molecular weight compounds, acidic and volatile in nature, with strong absorption property in UV/IR region. A matrix is used to overlay the bacterial (or fungal) colony spot on the MALDI-TOF MS target plate prior to analysis. Different types are available in the market for use with MALDI-TOF MS, with different properties and applications.

Simple protein extraction (using either formic acid or ethanol-acetonitrile) has been demonstrated to be cost-effective, fast and significantly enhances the ability of MALDI-TOF MS to correctly identify organisms[5].

The concentrated form of formic acid is highly corrosive therefore appropriate personal protective clothing must be worn at all times when in use. Extreme care must be taken by persons using this reagent. It should be noted that formic acid has specific toxic effects to humans; optic nerve damage, kidney damage and skin allergy that manifests upon re-exposure to the chemical. Some chronic effects of formic acid exposure have been documented.

Ethanol can be used for extraction of pure colonies for MALDI-TOF MS analysis. Ethanol is an extremely flammable liquid. Direct contact of the eyes may cause irritation, redness, pain, corneal inflammation and possible corneal damage. Repeated or prolonged contact of skin may result in cracking, and possible secondary infection.

Acetonitrile can be used for extraction of pure colonies for MALDI-TOF MS analysis. Acetonitrile is a flammable liquid and vapour. It is toxic when inhaled or absorbed through the skin. It may cause damage to the following target organs: blood, kidneys, lungs, liver, mucous membranes, gastrointestinal tract, cardiovascular system, upper respiratory tract, eyes, skin and central nervous system (CNS). It may be harmful if swallowed.

Follow manufacturer's instructions on how to use the matrices and the chemicals mentioned above as some are associated with significant occupational hazards such as eye, skin and respiratory toxicity. In event of any exposure occurring, medical advice should be sought.

Refer to the current guidance on the safe handling of all organisms documented in the other SMIs.

The above guidance should be supplemented with local COSHH and risk assessments.

Compliance with postal and transport regulations is essential.

2 Reagents and equipment

MALDI-TOF MS plates:

There are two types of MALDI-TOF MS target plates; ready-to-use disposable and reusable MALDI target plates. Both offer the same level of performance. The main difference is that the latter requires thorough cleaning and checking after each use to avoid contamination of clinical samples/isolates tested.

Wooden or plastic stick for inoculation or

Pipette tips or

Disposable loops

MALDI-TOF MS equipment and extraction kits:

Depending on the equipment and extraction kits that are being used, follow manufacturer's instructions on use.

There are two main approaches;

- pure colonies on appropriate agar medium or plate. It is recommended that freshly grown colonies (grown overnight) should be used or in the case of slow-growing bacteria, grown for several days. Plates should not be kept at 4°C prior to use because it affects the quality of spectra which deteriorates relatively quickly within a couple of days. Storing the plates at room temperature for several days is acceptable
- clinical specimens (eg direct blood culture material, urine, cerebrospinal fluid (CSF), or protein extract) can be used. There are commercial extraction kits for use directly on positive blood culture bottles. All reagents and consumables required for processing blood culture fluid are supplied in the kit

3 Quality control organisms

Positive control

Bacterial test standard – supplied by manufacturer, used for daily calibration and is a control for each run/matrix. This can also be used to trend selected calibration peaks to detect early drift before the calibration fails.

Negative control

Running a blank spot with matrix supplied by manufacturer. This is done only to verify that the target plate has been properly cleaned if a reusable type is used.

Note:

The quality control organisms used is dependent on what the manufacturer provides. Follow manufacturer's instructions. Laboratories should include their own validated positive and negative controls strains when performing MALDI-TOF MS runs.

It should be noted that it is good practice not to use the same target position for either the positive or negative controls all the time.

4 Procedure and results[30]

A pure bacterial colony (typically single) is picked from a culture plate using a wooden or plastic stick, pipette tip, or loop and then is placed onto a spot on a MALDI-TOF MS target plate. This is known as a 'direct' smear application. Most bacteria will be identified readily with a direct smear application (without any requirement for formic acid overlay). Fungal colonies require extraction as previously mentioned in 'Extraction methods' (in the technical limitations section) before being tested.

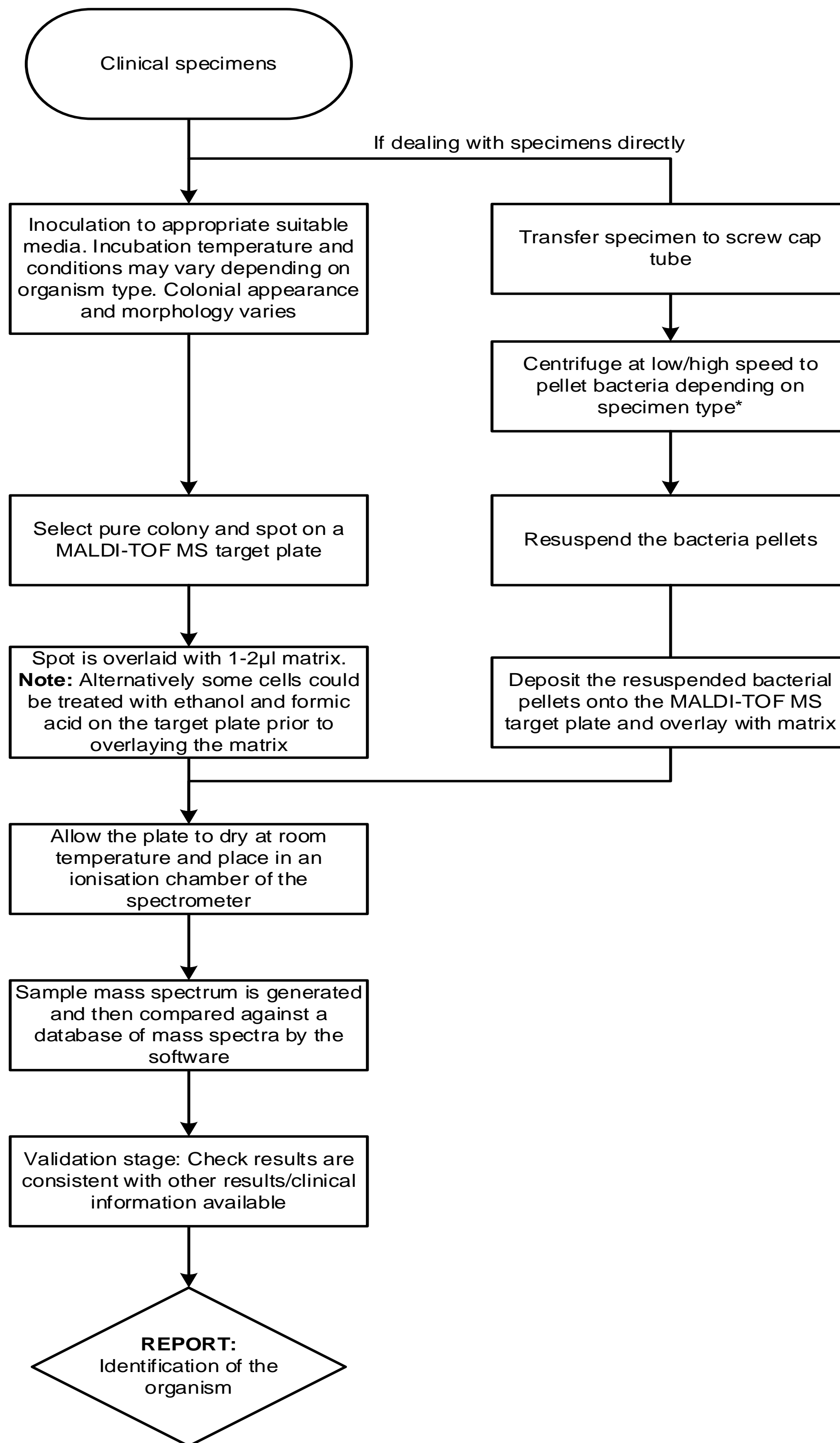
Note: Direct on-plate testing must be avoided with organisms hazardous to laboratory staff (for example, *Mycobacterium* species, *Brucella* species and *Bacillus anthracis*). The hazard group 3 organisms must be deactivated and then treated with formic acid overlay as it kills most bacteria. Any high risk organism should undergo extraction at the appropriate containment level before testing. This is done so as to avoid the risk of causing infection in staff handling these organisms[19]. It should also be noted that neither culture medium, incubation temperature and conditions, nor length of incubation affect the accuracy of identification.

The spot on the target plate is then overlaid with 1–2µL of matrix. Alternatively, where initial direct spot testing attempts fail, the bacterial cells can be overlaid with formic acid before the matrix is added. Fungal cells could be treated with ethanol and formic acid on the target plate prior to overlaying with matrix. This is often referred to as "On Target Lysis". The matrix should be applied within a short time frame to prevent oxidisation of the sample on the target plate.

Following a short drying period at room temperature, the plate is placed in the ionisation chamber of the mass spectrometer for analysis.

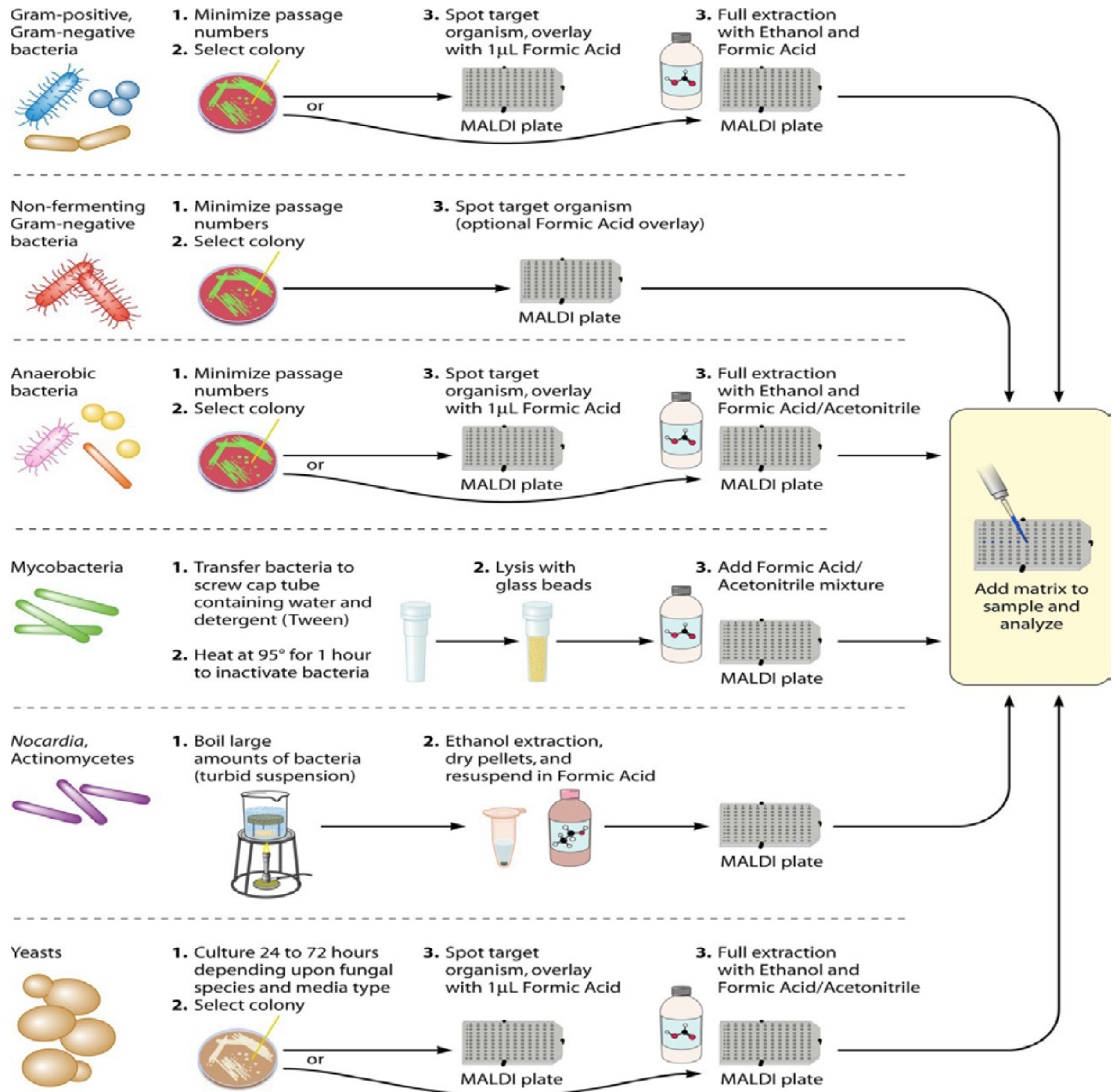
A mass spectrum is generated and automatically compared against a database of mass spectra by the software, resulting in identification of the organism. Users must follow the recommendations given from the manufacturers regarding when the identification provided can be regarded as satisfactory at either species or genus level[5].

Appendix 1: MALDI-TOF MS flowchart



This flowchart is for guidance only.

Appendix 2: Summary of examples for MALDI-TOF MS sample preparations for use with different classes of organisms



"On target lysis"

(Courtesy of Andrew E. Clark et al 2013) [5]

Note: It should be noted that some Gram positive and Gram negative bacteria can either be processed directly without the need for addition of formic acid. Proper biological safety precautions should be followed and most especially with respect to dangerous members of these groups of organisms. Details on how to process clinical specimens for MALDI-TOF MS analysis can be viewed in the reference above.

References

Modified GRADE table used by UK SMIs when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMIs for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VI). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Strength of recommendation	**Quality of evidence**
A Strongly recommended	I Evidence from randomised controlled trials, meta-analysis and systematic reviews
B Recommended but other alternatives may be acceptable	II Evidence from non-randomised studies
C Weakly recommended: seek alternatives	III Non-analytical studies, eg case reports, reviews, case series
D Never recommended	IV Expert opinion and wide acceptance as good practice but with no study evidence
	V Required by legislation, code of practice or national standard
	VI Letter or other

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3. Welker M, Moore ER. Applications of whole-cell matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry in systematic microbiology. SystApplMicrobiol 2011;34:2-11. **A, III**

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6. Hrabak J, Chudackova E, Walkova R. Matrix-assisted laser desorption ionization-time of flight (maldi-tof) mass spectrometry for detection of antibiotic resistance mechanisms: from research to routine diagnosis. ClinMicrobiolRev 2013;26:103-14. **B, III**

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