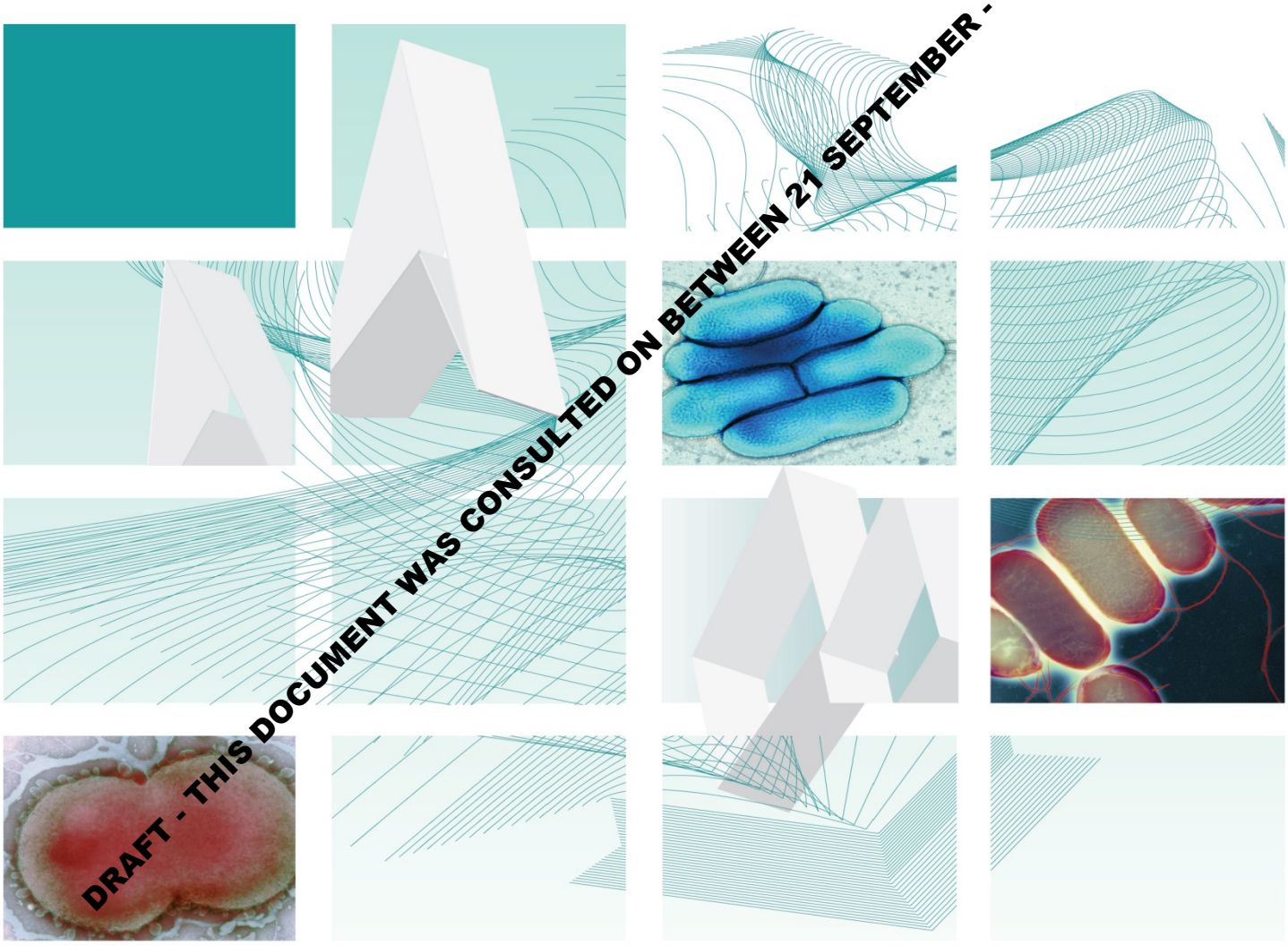
Public Health England

Protecting and improving the nation's health

UK Standards for Microbiology Investigations

Matrix-Assisted Laser Desorption/Ionisation - Time of Flight Mass Spectrometry (MALDI-TOF MS) Test Procedure



NICE accredited
www.nice.org.uk/accreditation

NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology – Test Procedures | TP40| Issue no: dzg+ |Issue date: dd.mm.yy <tab+enter> | Page: 1 of 21

Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

For further information please contact us at:

Standards Unit
Microbiology Services
Public Health England
61 Colindale Avenue
London NW9 5EQ

E-mail: standards@phe.gov.uk

Website: https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

PHE Publications gateway number: 2015003

UK Standards for Microbiology Investigations are produced in association with:

Logos correct at time of publishing.

Contents

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.	New amendment number/dd.mm.yy <tab+enter>
Issue no. discarded.	
Insert Issue no.	
Section(s) involved	**Amendment**

UK Standards for Microbiology Investigations[#]: scope and purpose

Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK.
- SMIs 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.
- SMIs 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-laboratories. 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.

[#]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.

Matrix-Assisted Laser Desorption/Ionisation - Time of Flight Mass Spectrometry (MALDI-TOF MS) Test

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 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 are 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

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, 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 to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time 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.

Suggested citation for this document

Public Health England. (YYYY <tab+enter>). Matrix-Assisted Laser Desorption/Ionisation - Time of Flight Mass Spectrometry (MALDI-TOF MS) Test . UK Standards for Microbiology Investigations. TP 40 Issue d. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

Scope of document

This SMI describes the test procedure, MALDI-TOF MS and its use in the examination of 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 Q1 - Commercial and in-house diagnostic tests: evaluations and validations.

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, 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. 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 several days. With prior knowledge of microorganism type (through using conventional and supplemental tests); users find it easier to know whether a bacterium or yeast is being tested. Without this, identification attempts usually fail. 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[1].

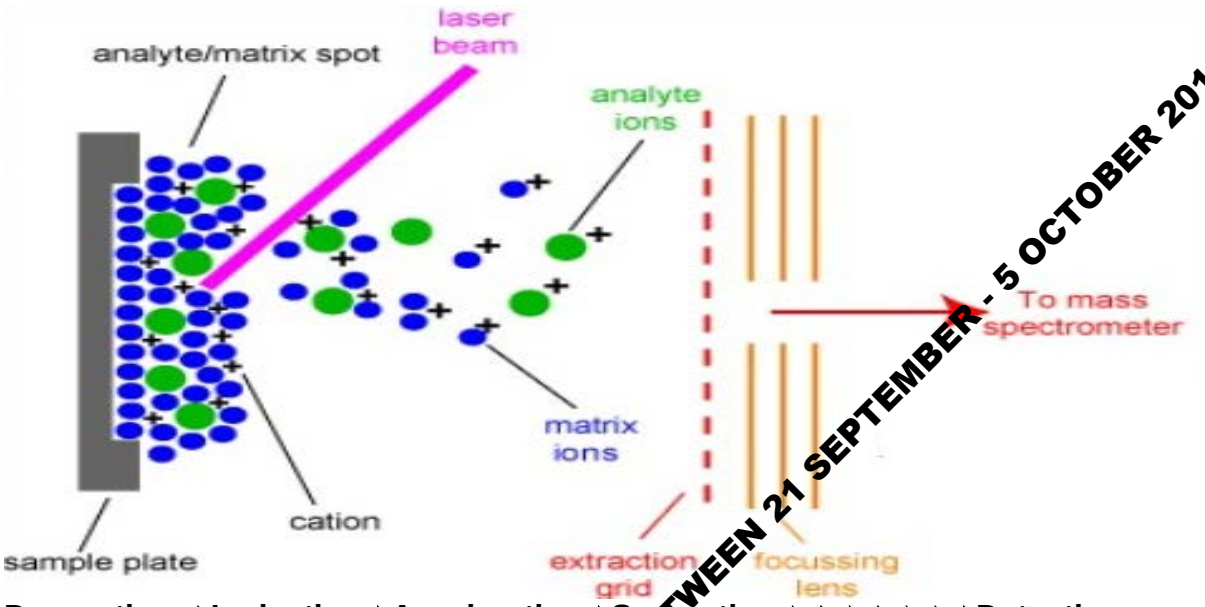
Ultimately, MALDI-based identification systems may prove the most cost-effective means of identification dependent only on how comprehensive the databases are[2] and the through-put of samples to warrant the initial substantial capital outlay. Another drawback is with the currently available commercial platforms. A number of well-established commercial manufacturers use their own algorithms, databases, software, and interpretive criteria for microbial identification, thereby making numerical data (ie, spectral scores) between these different commercial systems not directly comparable.

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[3].

This method has been used successfully in the identification of bacteria and fungi.

Mechanism of MALDI-TOF MS



Desorption ⇨Ionisation⇨Acceleration⇨Separation⇨⇨⇨⇨⇨⇨⇨Detection

Adapted from University of Bristol

The mechanism 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. They are as follows:

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[4,5].

Differentiation between organisms

Another 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. Direct discrimination between strains such as Meticillin resistant *S. aureus* and Meticillin sensitive *S. aureus* strains is a great challenge for MALDI-TOF MS applications in diagnostic laboratories[6].

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[7,8].

Existing taxonomical databases

MALDI-TOF 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[3]. However, this requires a confident molecular 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* spp, *Pandoraea pulmonicola* misidentified as *Sphingobacterium spiritivorum* [9-12].

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 organisms are between *Streptococcus pneumoniae* and *Streptococcus mitis* as well as most strains of *Haemophilus influenzae* and *Klebsiella pneumoniae*[9]. The key to overcoming some of these limitations lies in the quantity of inoculum and extraction method used.

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)[13]. This maximises the chances of having a reportable result without

further identification attempts and might be particularly valuable with mucoid colonies[14]. If testing in duplicate is used, the user needs to have a "reconciliation strategy"[7,8,15]. There must be an explicit reconciliation strategy in the local SOPs.

Commercial platforms

Another drawback is with the currently available commercial platforms. A number of well-established commercial manufacturers use their own algorithms, databases, software, and interpretive criteria for microbial identification, thereby making numerical data (that is, spectral scores) between these different commercial systems not directly comparable[3,4].

Note: Users should be warned that some MALDI-TOF MS commercial software might not be able to identify Hazard Group 3 pathogens unless the user applies with the commercial company for the full database, which will not normally be supplied due to bioterrorism concerns[3]. This could also potentially lead to additional testing of these isolates for further identification, thereby increasing the potential for exposure to laboratory staff.

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[4,9].

Other 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[3,4].

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. 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; it offers the potential to detect carbapenemase production, however, further improvements and validation are required before antibiotic susceptibility testing becomes routine practice[4,6,16-18].

Direct testing of clinical specimens

This technology has 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. 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 will as improvements in the databases will be required prior to implementation

in clinical laboratories that will 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[4,19].

Other limitations may include laboratories suffering financial loss on existing equipment due to acquiring the equipment needed for MALDI-TOF MS.

Extraction methods

There are several extraction methods used in MALDI-TOF MS for the pre-treatment of clinical specimens/ isolates[4].

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[9]. Filamentous fungi still lack standardised extraction protocols and commercially available databases lack the full scope required for fungal identification.

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[20].

For more information on the different recommended suggestions for MALDI-TOF MS sample preparations for use with different classes of organisms, see appendix 2[4].

Misidentifications

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[3].

Other strengths of MALDI-TOF MS

MALDI-TOF MS has other strengths. They include:

- it requires only a single colony 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
- this technique is green and has a small carbon footprint
- requires minimal consumables
- useful in identification of bacteria that are difficult to culture such as Mycobacteria, *Bartonella* species, *Legionella* species, etc[21]
- 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[22,23]
- useful in the identification of clinically relevant yeasts such as *Candida* species[24]

- minimal training required

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 away.

The performance of the extraction step and of the MALDI-TOF mass spectrometer may be checked by routinely testing a few selected bacterial strains, 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 get accurate results. This should be done more frequently if equipment is heavily used or located in a dusty or crowded area[9].

IT and reports

Microbiology laboratories send important results to the medical microbiologists for validation and comments, before release to the users. With increasing centralisation, many microbiologists no longer have a microbiology laboratory on site and are entirely reliant on the information provided with the report, in order to decide whether further or different identification procedures are required. 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 without or with extraction and the log scores that define the probability of an accurate identification. When there is no interface, the details of the method use, the number of the attempts and the log scores should be entered manually at least for the potentially “problematic” identifications. No identification system achieves 100% accuracy and the MALDI-TOF MS technique has a number of known weaknesses: the medical microbiologists must 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[25-41]

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 eg 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.

Matrices are low molecular weight compounds, acidic and volatile in nature, with strong absorption property in UV/IR region. Different types of matrices are available in the market for use with MALDI-TOF MS, with different properties and applications.

Formic acid can be used for preparation of clinical samples for MALDI- TOF MS analysis. The concentrated form 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.

Follow manufacturer's instructions on how to use both the matrices and formic acid as some are associated with significant occupational hazards such as eye, skin and respiratory toxicity.

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

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 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

OR

- 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 clinical specimens – blood culture broths. 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.

Negative control

Running a blank spot with matrix supplied by manufacturer, only to verify that the target plate has been properly cleaned.

Note: The quality control organisms used is dependent on what the manufacturer provides. Follow manufacturer's instructions.

4 Procedure and results[14]

- A pure bacterial colony (typically single) is picked from a culture plate to a spot on a MALDI-TOF MS target plate using a wooden or plastic stick, pipette tip, or loop which is known as a direct smear application[a]. Fungal colonies require extraction as previously mentioned in extraction method (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[5]. 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, bacterial (where after initial direct spot testing attempts fail, the bacterial cells can be overlaid with formic acid/ acetonitrile before the matrix is added) or fungal cells could be treated with ethanol and formic acid / acetonitrile 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

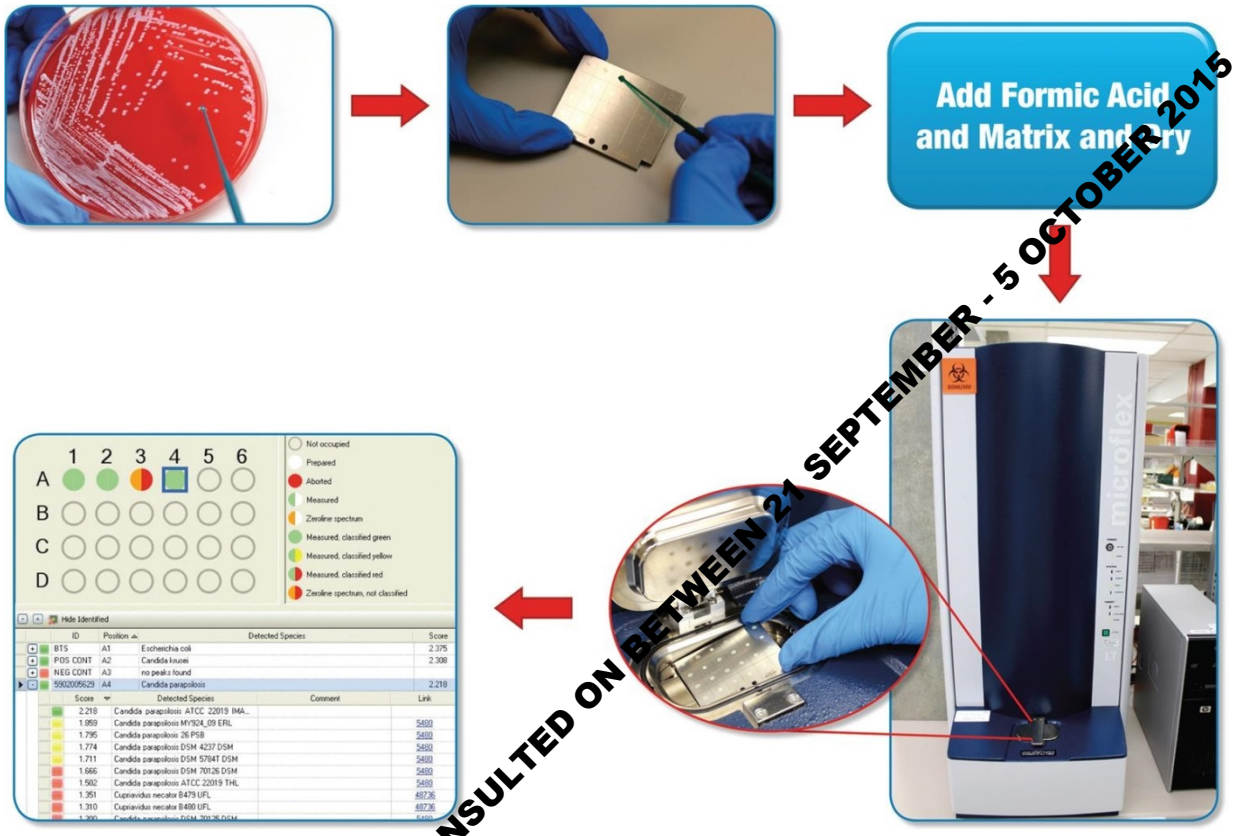
- A mass spectrum is generated and automatically compared against a database of mass spectra by the software, resulting in identification of the organism. Users must follow the recommendations given from the manufacturers regarding when the identification provided can be regarded as satisfactory at either species or genus level[4]

Matrix-Assisted Laser Desorption/Ionisation - Time of Flight Mass Spectrometry (MALDI-TOF MS) Test

Footnote:

a. Most bacteria will identify readily with a direct smear application (without any requirement for formic acid overlay.

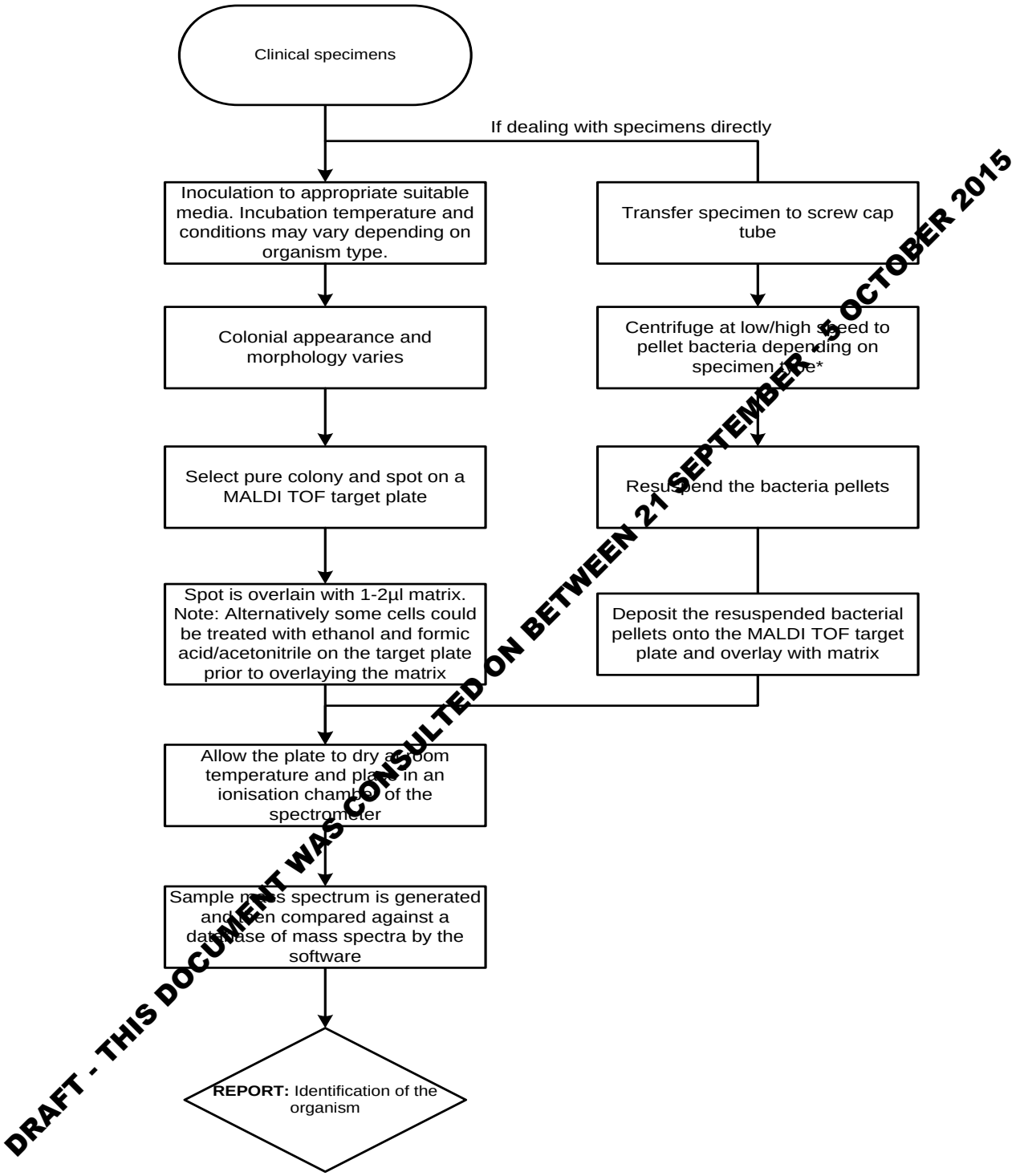
An example of MALDI-TOF MS illustration workflow (*Courtesy of Robin Patel*)[3].



It should be noted that not all isolates should be treated with formic acid prior to the addition of the matrix.

This MALDI-TOF MS workflow is for guidance only.

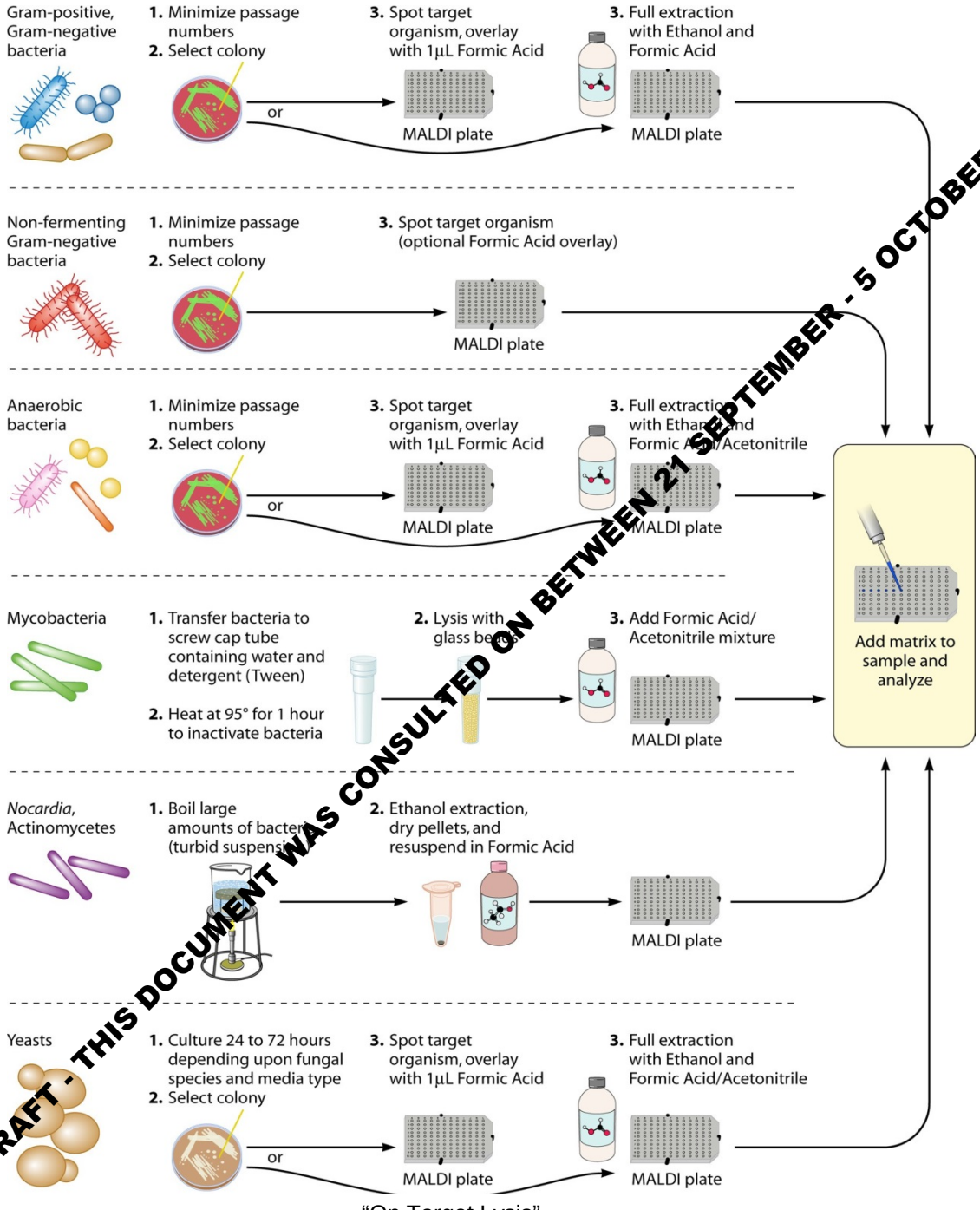
Appendix 1: MALDI-TOF MS flowchart



* If processing blood specimen for MALDI TOF use the appropriate kit and follow manufacturers instructions

This flowchart is for guidance only.

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



"On Target Lysis"

(*Courtesy of Andrew E. Clark et al 2013*) [4]

Note: Proper biological safety precautions should be followed and most especially with respect to dangerous members of these groups of organisms.

References

1. Barbuddhe SB, Maier T, Schwarz G, Kostrzewa M, Hof H, Domann E, et al. Rapid identification and typing of listeria species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Appl Environ Microbiol 2008;74:5402-7.

2. Welker M, Moore ER. Applications of whole-cell matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry in systematic microbiology. Syst Appl Microbiol 2011;34:2-11.

3. Patel R. Matrix-assisted laser desorption ionization-time of flight mass spectrometry in clinical microbiology. Clin Infect Dis 2013;57:564-72.

4. Clark AE, Kaleta EJ, Arora A, Wolk DM. Matrix-assisted laser desorption ionization-time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology. Clin Microbiol Rev 2013;26:547-603.

5. Mather CA, Rivera SF, Butler-Wu SM. Comparison of the Bruker Biotyper and Vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry systems for identification of mycobacteria using simplified protein extraction protocols. J Clin Microbiol 2014;52:130-8.

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. Clin Microbiol Rev 2013;26:103-14.

7. van Veen SQ, Claas EC, Kuijper EJ. High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. J Clin Microbiol 2010;48:900-7.

8. Moussaoui W, Jaulhac B, Hoffmann AM, Ludes B, Kostrzewa M, Riegel P, et al. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry identifies 90% of bacteria directly from blood culture vials. Clin Microbiol Infect 2010;16:1631-8.

9. Croxatto A, Prod'hom G, Greub G. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. FEMS Microbiol Rev 2012;36:380-407.

10. Richter SS, Sercia L, Branda JA, Burnham CA, Bythrow M, Ferraro MJ, et al. Identification of Enterobacteriaceae by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using the VITEK MS system. Eur J Clin Microbiol Infect Dis 2013;32:1571-8.

11. Branda JA, Markham RP, Garner CD, Rychert JA, Ferraro MJ. Performance of the Vitek MS v2.0 system in distinguishing Streptococcus pneumoniae from nonpneumococcal species of the Streptococcus mitis group. J Clin Microbiol 2013;51:3079-82.

12. McElvania TE, Burnham CA. Evaluation of the Bruker Biotyper and VITEK MS MALDI-TOF MS systems for the identification of unusual and/or difficult-to-identify microorganisms isolated from clinical specimens. Eur J Clin Microbiol Infect Dis 2014;33:2163-71.

13. Powell EA, Blecker-Shelly D, Montgomery S, Mortensen JE. Application of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of the fastidious pediatric pathogens Aggregatibacter, Eikenella, Haemophilus, and Kingella. J Clin Microbiol 2013;51:3862-4.

14. Marko DC, Saffert RT, Cunningham SA, Hyman J, Walsh J, Arbefeville S, et al. Evaluation of the Bruker Biotyper and Vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry systems for identification of nonfermenting gram-negative bacilli isolated from cultures from cystic fibrosis patients. J Clin Microbiol 2012;50:2034-9.

15. Tan KE, Ellis BC, Lee R, Stamper PD, Zhang SX, Carroll KC. Prospective evaluation of a matrix-assisted laser desorption ionization-time of flight mass spectrometry system in a hospital clinical microbiology laboratory for identification of bacteria and yeasts: a bench-by-bench study for assessing the impact on time to identification and cost-effectiveness. J Clin Microbiol 2012;50:3301-8.

16. Burckhardt I, Zimmermann S. Using matrix-assisted laser desorption ionization-time of flight mass spectrometry to detect carbapenem resistance within 1 to 2.5 hours. J Clin Microbiol 2011;49:3321-4.

17. Hrabak J, Studentova V, Walkova R, Zemlickova H, Jakubu V, Chudackova E, et al. Detection of NDM-1, VIM-1, KPC, OXA-48, and OXA-162 carbapenemases by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2012;50:2441-3.

18. Sparbier K, Schubert S, Weller U, Boogen C, Kostrzewa M. Matrix-assisted laser desorption ionization-time of flight mass spectrometry-based functional assay for rapid detection of resistance against beta-lactam antibiotics. J Clin Microbiol 2012;50:927-37.

19. Kok J, Thomas LC, Olma T, Chen SC, Iredell JR. Identification of bacteria in blood culture broths using matrix-assisted laser desorption-ionization Sepsityper and time of flight mass spectrometry. PLoS One 2011;6:e23285.

20. Fournier R, Wallet F, Grandbastien B, Dubreuil L, Courcol R, Neut C, et al. Chemical extraction versus direct smear for MALDI-TOF mass spectrometry identification of anaerobic bacteria. Anaerobe 2012;18:294-7.

21. Biswas S, Rolain JM. Use of MALDI-TOF mass spectrometry for identification of bacteria that are difficult to culture. J Microbiol Methods 2013;92:14-24.

22. Garner O, Mochon A, Branda J, Burnham CA, Bythrow M, Ferraro M, et al. Multi-centre evaluation of mass spectrometric identification of anaerobic bacteria using the VITEK(R) MS system. Clin Microbiol Infect 2014;20:335-9.

23. Rychert J, Burnham CA, Bythrow M, Garner OB, Ginocchio CC, Jennemann R, et al. Multicenter evaluation of the Vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry system for identification of Gram-positive aerobic bacteria. J Clin Microbiol 2013;51:2225-31.

24. Westblade LF, Jennemann R, Branda JA, Bythrow M, Ferraro MJ, Garner OB, et al. Multicenter study evaluating the vitek MS system for identification of medically important yeasts. J Clin Microbiol 2013;51:2267-72.

25. European Parliament. UK Standards for Microbiology Investigations (SMIs) use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".

26. Official Journal of the European Communities. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices. 7-12-1998. p. 1-37.

27. Health and Safety Executive. Safe use of pneumatic air tube transport systems for pathology specimens. 9/99.

28. Department for transport. Transport of Infectious Substances, 2011 Revision 5. 2011.

29. World Health Organization. Guidance on regulations for the Transport of Infectious Substances 2013-2014. 2012.

30. Home Office. Anti-terrorism, Crime and Security Act. 2001 (as amended).

31. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive. 2013. p. 1-32

32. Advisory Committee on Dangerous Pathogens. Infections at work: Controlling the risks. Her Majesty's Stationery Office. 2003.

33. Advisory Committee on Dangerous Pathogens. Biological agents: Managing the risks in laboratories and healthcare premises. Health and Safety Executive. 2005.

34. Advisory Committee on Dangerous Pathogens. Biological Agents: Managing the Risks in Laboratories and Healthcare Premises. Appendix 1.2 Transport of Infectious Substances - Revision. Health and Safety Executive. 2008.

35. Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. MMWR Surveill Summ 2012;61:1-102.

36. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002. 5th ed. HSE Books; 2002.

37. Health and Safety Executive. Five Steps to Risk Assessment: A Step by Step Guide to a Safer and Healthier Workplace. HSE Books. 2002.

38. Health and Safety Executive. A Guide to Risk Assessment Requirements: Common Provisions in Health and Safety Law. HSE Books. 2002.

39. Health Services Advisory Committee. Safe Working and the Prevention of Infection in Clinical Laboratories and Similar Facilities. HSE Books. 2003.

40. British Standards Institution (BSI). BS EN12469 - Biotechnology - performance criteria for microbiological safety cabinets. 2000.

41. British Standards Institution (BSI). BS 5726:2005 - Microbiological safety cabinets. Information to be supplied by the purchaser and to the vendor and to the installer, and siting and use of cabinets. Recommendations and guidance. 24-3-2005. p. 1-14