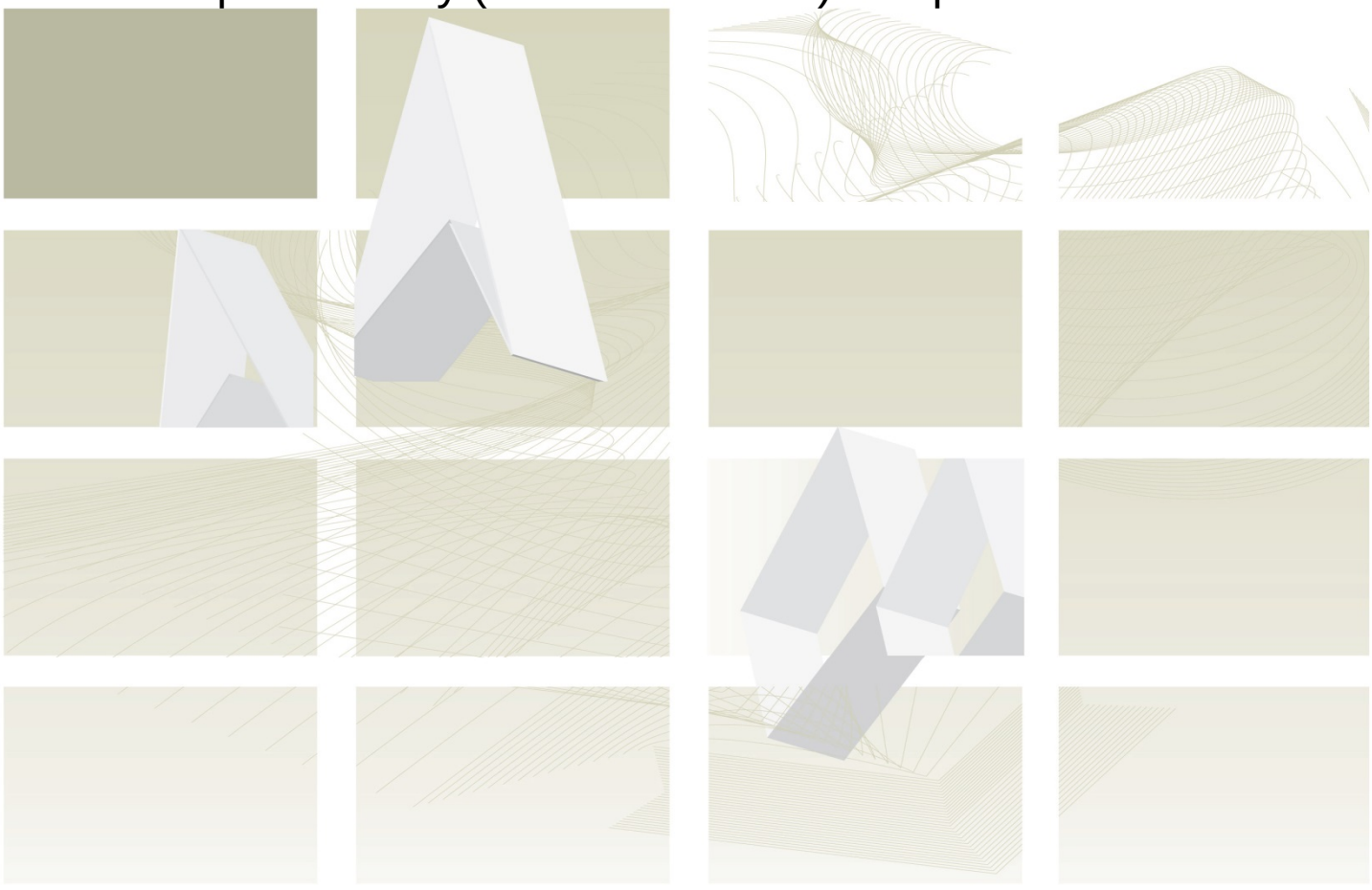
Public Health England

NHS

UK Standards for Microbiology Investigations

Review of users' comments received by
Working group for microbiology standards in clinical bacteriology

TP 40 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.

Recommendations are listed as ACCEPT/ PARTIAL ACCEPT/DEFER/ NONE or PENDING

1st Consultation: 10/04/2015 – 06/05/2015

Version of document consulted on: TP 40dm+

Proposal for changes

Comment number	1		
Date received	14/04/2015	**Lab name**	Microbiology-Belfast Health & Social Care Trust
Section	Introduction		
Comment			
The introduction of this SMI comments that users do not need to know if the target organism is a bacterium or yeast. As a user of the biomerieux Vitek MS system, knowledge of the organism is required in order to perform an extraction process. Without this, ID attempts usually fail.			
Recommended action	**ACCEPT** This has been updated in the document accordingly.		

Comment number	2		
Date received	24/04/2015	**Lab name**	Southwest Pathology Services
Section	Appendix 2		
Comment			
So far, we only have limited experience and comments are therefore based on anecdotal evidence. Most bacteria identify readily without any extraction, just requiring direct smearing of the colony onto the plate, without any requirement for formic acid overlay. However, *Strep. pneumoniae* and *Strep. mitis/oralis* strains do usually require formic acid overlay for a reliable identification. Yeast organisms usually identify more reliably with a full formic acid/acetonitrile extraction technique, but a simple formic acid overlay does sometimes work also.			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	**ACCEPT** This has been updated in the document accordingly.		

Comment number	3		
Date received	30/04/2015	**Lab name**	bioMerieux INC.
Section	Entire Document		
Comment			
Regarding the document entitled UK Standards for Microbiology Investigations Matrix-Assisted Laser Desorption/Ionisation - Time of Flight Mass Spectrometry (MALDI-TOF MS) Test Procedure with reference number TP 40 and PHE Publications gateway number 2015003, I have the following comments: a. In many areas of the document, it is specific to only one (Bruker Biotyper) of the two commercial MALDI-TOF platforms (Bruker Biotyper and bioMerieux VITEK MS) and can be misconstrued as the procedure applicable to both systems. In fact, the specimen preparation for the bioMerieux VITEK MS is much simpler and rarely requires complex extraction methods, i.e., examples in Appendix 2 are Bruker-specific and in nearly every case not relevant for bioMerieux VITEK MS only yeasts are subjected to on target extraction with formic acid only and never to a tube extraction; tube extractions on bioMerieux VITEK MS are performed only with moulds, mycobacteria, and *Nocardia*. b. I suggest that this document either be labelled as the Bruker MALDI-TOF procedure or prepared in concert with a bioMerieux VITEK MS user so that it can be made more generically acceptable. c. For your information, CLSI currently has a document preparation subcommittee working on a generic document that will be labelled M58. Perhaps you should contact CLSI to ensure unification of these documents. d. The limitations in performance on page 10 are Bruker-specific and not relevant for bioMerieux VITEK MS. e. The pictures on page 14 are Bruker-specific and not relevant for bioMerieux VITEK MS. f. Regarding your submission form and question 9: Misrepresentation of this Bruker-centric procedure as the generic procedure can pose health risks to patients since performance limitations for the groups mentioned on page 10 are not applicable to bioMerieux VITEK MS. Therefore, reliable results from the bioMerieux VITEK MS for very clinically relevant taxa such as *S. pneumoniae, K. pneumoniae, H. influenzae*, etc. might be ignored from MALDI-TOF and delayed by dependence on some alternate method due to this document and what is seen only with Bruker Biotyper and corroborated by the scientific literature.			
Financial barriers			
No.			
Health benefits			
Please see above.			
Recommended action	a. **NONE** The information in the document is a compilation of some		

	of the technical limitations/information that could be experienced by users when using the different platforms available. No specific platform has been mentioned or favoured over another as the UK SMIs suggest. All the different extraction methods that could be applied have also been mentioned without covering any specific platform. b. **NONE** This document is intended to be a generic guidance for all platforms. The technical limitations are a compilation of information that could be experienced by users when using the different platforms available. It is given so that users are aware of the different limitations that could arise from using any MALDI-TOF MS platform without mentioning any specific company product. c. **NONE** The UK SMI team have been in contact with CLSI who have advised us that their document is still being developed and not yet published. However, UK SMIs are developed independently of other organisations as they are intended to satisfy the requirements of laboratories in the UK. d. **NONE** The technical limitations are not written specifically for any platform. This general information is a compilation of information that could be experienced by users when using the different platforms available. It is given so that users are aware of the different limitations that could arise from using any MALDI-TOF MS platform without mentioning any specific company product. e. **ACCEPT** This diagram has been removed from the document. f. **NONE** Same as response in comment 1 and 2. These comments have been discussed with BWG members and a reply letter will be written to the bioMerieux INC company.

Comment number	4		
Date received	04/05/2015	**Lab name**	MALDI biotyper Applications Lab manager and Scientist
Section	Formic acid overlay		

Comment
In terms of the new guidelines recommending the use of Formic acid 70% as a general first line, I feel is possibly not necessary. The addition of using 70% formic acid with every sample adds an additional RA for the general use of this within the department. However with the use of 70% FA sporadically can minimise its use on the general bench. If you have any questions at all about this, please do not hesitate to contact myself.
Financial barriers
No.
Health benefits
Yes.

Recommended action	**ACCEPT** Formic acid overlay is used in majority of laboratories that perform MALDI-TOF MS on clinical specimens. However, an email was written to MALDI biotyper Applications Laboratory Manager as the question was not clear. A reply was received and it said that it should be worth mentioning that not all isolates are treated with 70% formic acid, that some are identified with a direct smear application. This has been updated in the document accordingly.

Comment number	5		
Date received	06/05/2015	**Professional body**	Institute of Biomedical Science
Section	Various		

Comment

Introduction

a. "Users do not even need to know whether a bacterium or yeast is being tested".

   It is felt that this is an unhelpful statement, in reality you should have some idea of what the organism is and you have to be 100% confident that your database is complete (which none are) and you have not got a mixture of organisms. MALDI ID should always be backed up with supplementary tests.

b. "may prove the most cost-effective means of identification dependent only on how comprehensive the databases are[2]."

   It was noted that it is only cost effective if you have the through-put of samples to warrant the initial substantial capital outlay. The relationship of volume to cost-effectiveness should be stated.

Technical Information/Limitations

c. Differentiation between organisms

Problems related to differentiation between genera is documented, however there is not a section for species level limitations. References (7) and (8) cited in the draft document both discuss many problematic areas. The following organisms should be highlighted in the narrative:

Mycobacteria

Burkholderia

Acinetobacter

Corynebacteria

Beta haemolytic streptococci

d. Difficulty in lysing cell wall structures

This section states that if testing in duplicate is used, the user needs to have a 'reconciliation strategy'. References (7) and (8) are provided as evidence of this statement. There is no mention of a reconciliation strategy or what it entails in either of the references given. There needs to be further clarification of this point.

e. Identification of antimicrobial resistance

Reference (8) by Clark et al provides details related to the current limitations for detecting specific resistance mechanisms. It would be useful if a small section discussing the points raised by Clark and his team could be inserted into the narrative. The policy currently only mentions detection of methicillin; it is suggested that there should be scope to add details of beta lactamase testing at least. The SMI authors have made it clear that further improvements are required in specimen processing prior to implementation of direct testing of clinical samples however they have not considered that further improvements are required before antibiotic testing becomes routine practice.

Procedure and Results

f. First Point

A bacterial or fungal 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

Note: Direct on-plate testing must be avoided with organisms hazardous to laboratory staff (for example, *Brucella* species and *Bacillus anthracis*). This must be extracted with formic acid overlay as it kills most bacteria. This is done so as to avoid the risk of causing infection in staff handling these organisms.

It was noted that this point could be confusing as all HG3 organisms should be deactivated at CL3 before being put on to the target plate. It was also felt that it would be useful to document here that neither culture medium, incubation temperature, incubation conditions, nor length of incubation affect the accuracy of identification (also stated in reference (7) by van Veen et al).

g. Second Point

The spot on the target plate is then overlaid with matrix……

It would be helpful to the user to specify that matrix has to been applied within a short time frame to prevent oxidisation of the sample on the target plate.

Recommended	a. **ACCEPT**

action	This has been updated in the document accordingly. b. **ACCEPT** This has been updated in the document accordingly. c. **ACCEPT** This has been updated in the document accordingly. d. **NONE** The references clearly discuss reconciliation strategy when testing in duplicates. e. **ACCEPT** This has been updated in the document accordingly. f. **ACCEPT** This has been updated in the document accordingly. g. **ACCEPT** This has been updated in the document accordingly.

Comment number	6		
Date received	05/05/2015	**Professional body**	UK CMN
Section	Various		
Comment			
The UK CMN have sent a track version of this SMI with various suggestions.			
Recommended action	**ACCEPT** Most of the comments have been accepted and updated accordingly.		

2nd Consultation: 21/09/2015 – 05/10/2015

Version of document consulted on: TP 40dzg+

Proposal for changes

Comment number	1		
Date received	23/09/2015	**Lab name**	Bristol PHE Lab
Section	3 QC Organisms, 3 - Procedure		
Comment			
a. I would suggest that the QC includes the running of a Gram positive NCTC/ATCC control and a Gram negative NCTC/ATCC control as well as the negative control of the matrix only. b. Also it is good practice not to use the same target position for either the positive or negative controls. The negative control is a good test on how well the re-usable			

ones have been cleaned and needs to control the whole target not just the same positions. The BTS is not necessarily used for DAILY calibration (may depend on manufacturer. Need to include the need to trend selected calibration peaks to detect early drift before the calibration fails. c. In section 4 the pictures clearly relate to the Bruker Maldi-TOF so maybe commercial endorsement inferred?! d. Also, just another general thought - would it be useful in future to give details of EQA schemes available for the various SOPs where relevant - eg MALDI currently a pilot scheme run by QCMD, currently no EQA for crypto/giardia EIA (I know one is being developed by NEQAS) or for TV / BV in genital samples.	
Financial barriers	
No.	
Health benefits	
No.	
Recommended action	a. **ACCEPT** This has been updated in the document accordingly. b. **ACCEPT** This has been amended in the document accordingly. c. **NONE** The picture has been updated with appropriate pictures. d. **NONE** The information is not in line with the UK SMI document.

Comment number	2		
Date received	24/09/2015	**Lab name**	Bruker UK Limited
Section	Various		
Comment			
a. Technical information/limitations: Difficulty in lysing cell wall structures Are these isolates really difficult to direct smear? I am not so certain of these. b. Technical information/limitations: Commercial platforms Please note that the Vitek MS does not even have the capability to ask the manufacturers for the SR database they simply do not have the isolates c. Technical information/limitations: Identification of antimicrobial resistance Bruker have IP on the detection of carbapenemases			
Recommended action	a. **NONE** There are many journals/evidence to show that some organisms possess capsules which prevent efficient lysis		

	of cells. b. **NONE** Many thanks for the information. c. **NONE** Many thanks for the information. Two more recent references have been added to this section.

Comment number	3		
Date received	28/09/2015	**Lab name**	Animal and Plant Health Agency
Section	Various		
Comment			
a. In appendix 2 there is no mention of overlaying samples with matrix, after overlaying with formic acid or doing an extraction. This is included in appendix 1, and perhaps it is felt this does not need to be repeated it in appendix 2, but if appendix 2 only was used as a guide, the procedure would not work without matrix. b. With respect to the comments in the document that "Most bacteria will identify readily with a direct smear application (without any requirement for formic acid overlay), we would agree with this from our work, but we have shown overlay with formic acid usually increases the score, and as such can push a confidence level up from genera only to species. This might be important in some situations and could be mentioned. c. In the section on culture medium, I suggest the document recommends a simple non-selective media such as blood agar or nutrient agar (no recommendation is made). As standard, if possible, we take isolates from blood agar before identification. Although we have not done extensive work, incidental results suggest that some selective agars can reduce the scores obtained, and the document suggest similar, but without recommending any agars. d. Under section 3 "Quality control organism" the document only mentions the control standard. Whilst in general this is all we have done up till now, it might be good to add a sentence here that laboratories should also include ~2 to 4 control stains of most relevance to their laboratory, and future work that we do will include further controls. e. For a clinical laboratory doing direct identification from clinical samples such as urine, it might be good to have a positive control from a spiked sample with a known count of bacteria to show how sensitive the method is for that lab? Without such a positive control the threshold of detection would I think be uncertain?		
Recommended action	a. **NONE** This is already in the appendix diagram. b. **NONE** Many thanks for the information.		

	c. **NONE** This document does not recommend any selective agar in particular. Laboratories should validate whichever media they want to use before using routinely. However, the technical limitations lists what should be considered when using selective agar such as CNA agar plate. d. **ACCEPT** This has been updated in the document accordingly. e. **NONE** Many thanks for the information. However, laboratories that wish to use positive control from a spiked sample should validate this according their local validation policy.

Comment number	4		
Date received	28/09/2015	**Lab name**	Microbiology, Medical Faculty of Univ. Rovira i Virgili
Section	Introduction, but I have other suggestions that are mark in the pdf text that I attach here		
Comment			
In fact MALDI TOF relay mainly on ribosomal proteins but nothing of this is mentioned and to some extent the species that show a high similarity of their 16S rRNA gene are more difficult to differentiate with this method.			
Evidence			
Appl Microbiol Biotechnol. 2015 Jul;99(13):5547-62. doi: 10.1007/s00253-015-6515-3. Epub 2015 Mar 18. Ribosomal protein biomarkers provide root nodule bacterial identification by MALDI-TOF MS. Ziegler D1, Pothier JF, Ardley J, Fossou RK, Pfluger V, de Meyer S, Vogel G, Tonolla M, Howieson J, Reeve W, Perret X. J Microbiol Methods. 2013 Sep;94(3):390-6. doi: 10.1016/j.mimet.2013.07.021. Epub 2013 Aug 3.Ribosomal proteins as biomarkers for bacterial identification by mass spectrometry in the clinical microbiology laboratory. Suarez S1, Ferroni A, Lotz A, Jolley KA, Guerin P, Leto J, Dauphin B, Jamet A, Maiden MC, Nassif X, Armengaud J.			
Financial barriers			
No.			
Health benefits			
The benefits are already stated.			
Recommended	**NONE**		

action	The Working Group for Standards in Clinical Bacteriology concluded that the reference by Ziegler D. et al should not be used as it was related to root nodule (soil) bacteria and not clinical specimens. The paper by Suarez is cited in the document accordingly.

Comment number	5		
Date received	29/09/2015	**Lab name**	Ninewells Microbiology
Section	4 & appendix 2		
Comment			
You seem to imply that all Gram positive and fermenting Gram negative bacteria need formic acid extraction. I am currently verifying the Maldi biotyper and I am not finding this to be necessary and this is not what is recommended by Bruker.			
Evidence			
I could provide all the runs that I have performed without formic acid if necessary.			
Financial barriers			
No.			
Health benefits			
No.			
Recommended action	**NONE** Appendix 2 clearly states that Gram positive and Gram negative bacteria can either be processed overlaid with formic acid or may need ethanol and formic acid extraction. Non-fermenting Gram negative bacteria are extracted directly and the use of formic acid overlay is optional. A caveat has been added in the appendix 2 under the note section that some Gram positive and Gram negative bacteria can either be processed directly without the need for addition of formic acid.		

Comment number	6		
Date received	30/09/2015	**Lab name**	MRC Toxicology Unit-Protein Profiling Group
Section	Extraction methods		
Comment			
My comment here would be on the statement that there is no best recommended			

extraction method---------- The concern here is that the different methods suggested in Appendix 2 is not very comprehensive and maybe should be expanded.
Financial barriers
No.
Health benefits
No substantial health effects from MALDI, provided GLP and Health and Safety protocols are followed, but obviously appropriate should be adopted when dealing highly pathogenic organisms.

Recommended action	**NONE** It is not within the scope of the UK SMI to recommend a best extraction method as it depends on the software used and for users to follow manufacturer's instructions during extraction and analysis using MALDI-TOF MS.

Comment number	7		
Date received	05/10/2015	**Lab name**	Microbiology Department, Royal Infirmary of Edinburgh
Section	Differentiation between organisms		

Comment
The document does not highlight that MALDI-TOF 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* which could have serious health, legal and social consequences. There is also evidence of *N.meningitidis* being mistakenly identified as a 'non-pathogenic' *Neisseria* sp. It would be prudent to include a statement to the effect of the guidance in the PHE SMI ID 6: Identification of pathogenic *Neisseria* species, which states under section 3.5.1.3 Identification tests available, the following: Therefore in sensitive or critical situations, confirmation of *Neisseria* species identification should be confirmed with phenotypic or molecular methods. Suitable alternative methods for identification may include API NH, VITEK 2 NH or rapid carbohydrate utilisation tests.
Evidence
Cunningham et al reported in Journal of Clinical Microbiology, March 2014 their experience of identifying 5 isolates of *Neisseria polysaccharea* (including a quality control strain *N. polysaccharea* ATCC 43768) as *N.meningitidis*, with MALDI-TOF scores suggesting reliable identification to species level. This is corroborated by previous data presented as an abstract by Vironneau et al in April 2013, describing misidentification of N*. polysaccharea* and *N.cinerea* as *N.meningitidis*. Deak et al reported a case in reference to Cunningham discussing their experience of blood culture isolate identified by their own institution as *Neisseria polysaccharea* subsequently confirmed by reference laboratory as *Neisseria meningitidis*. Further to this the PHE SMI ID 6: identification of

Neisseria species, last issued 26th June 2015 states-'Although the problem of the *Neisseria* genus study is complex, MALDI-TOF has been developed and validated to determine the clinically important species of *Neisseria* as *N. gonorrhoeae* and *N. meningitidis*, both are relatively straightforward to identify, the differences between many of the non-pathogenic strains are small and the speciation of these strains within a diagnostic setting is not always possible. While the identification of non-pathogenic *Neisseria* to species level is generally not required, the misidentification of these strains as *N. gonorrhoeae* or *N. meningitidis* can have serious health, legal and social consequences. Formal validation studies for MALDI-TOF MS of *N. gonorrhoeae* are limited. Therefore in sensitive or critical situations, confirmation of *Neisseria* species identification should be confirmed with phenotypic or molecular methods' Papers and documents attached in support.	
Financial barriers	
No.	
Health benefits	
No.	
Recommended action	**ACCEPT** This has been updated in the document accordingly.

Respondents indicating they were happy with the contents of the document

Overall number of comments: 1			
Date received	28/09/2015	**Lab name**	Royal Oldham Hospital