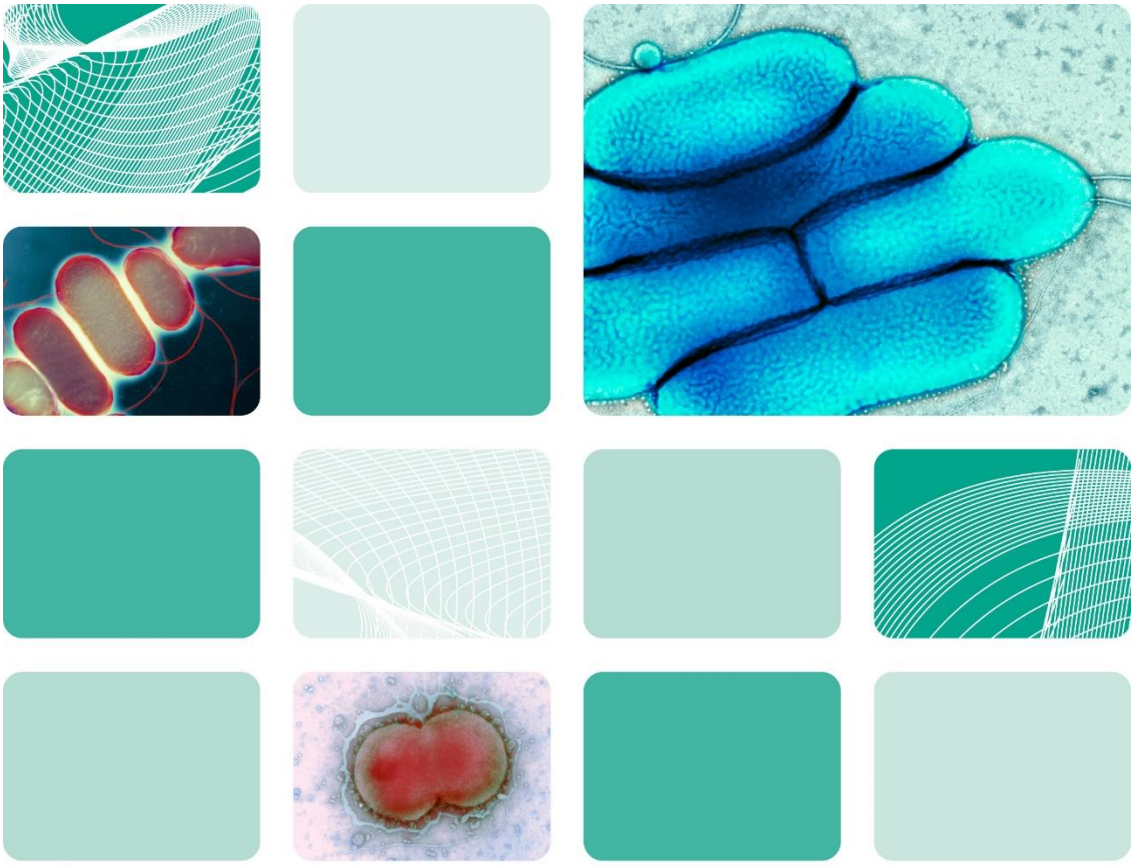
UK Health Security Agency

UK Standards for Microbiology Investigations

Identification of *Pseudomonas* species and other non-glucose fermenters



National Institute for Health and Care Excellence (NICE) has renewed accreditation of the process used by the **UK Health Security Agency** to produce **UK Standards for Microbiology Investigations (UK SMIs)**. The renewed accreditation is valid until **30 June 2026** and applies to guidance produced using the processes described in '**UK Standards for Microbiology Investigations Development Process' (2021)**. The original accreditation term began on 1 July 2011.

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Acknowledgments

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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.

UK SMIs are produced in association with:

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Contents

Amendment table

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

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	5/17.09.24
Issue number discarded	3
Insert issue number	4
Anticipated next review date*	17.09.24
Section(s) involved	**Amendment**
Whole document	The document has been transferred into a new template and headings have been reorganised. The hyperlinks in the document have been updated to direct the reader to UK SMI webpages hosted on the Royal College of Pathologists website. The information and references in the document have been updated.
Scope of document	The scope has been updated to reflect the focus of the document. Additional links have been added to UK SMI TP 40 and other identification documents for further information.
Introduction	Descriptions of each species and genera have been removed. Colonial morphology and microscopic appearance of relevant species have been summarised in table 1, section 8.2. Other relevant non-glucose fermenters have been listed.
Technical information	Some information has been moved to the relevant subheadings in section 8.
Safety considerations	References have been updated where available.
Identification	Subheadings have been restructured to reflect laboratory practices.

	Table 1 summarises microscopic and colonial appearance of relevant species. Table of oxidase test results added to biochemical tests.
Reporting	Information and subheadings have been updated.
Referral to reference laboratory	Hyperlinks have been updated.
Algorithm	Algorithm changed to reflect current laboratory processes. Results of oxidase test moved to section 8.
References	References updated where necessary.

*Reviews can be extended up to 5 years where appropriate

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

This UK Standards for Microbiology Investigations (UK SMI) document describes the identification of *Pseudomonas* species and other non-glucose fermenters associated with human infection from clinical specimens. It does not include identification from environmental samples.

It includes culture, Gram stain and matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for the identification of microorganisms. Some biochemical tests may not be performed routinely in laboratory except in cases where confirmation by an alternative technique is required or automated methods are not available.

The test procedure for MALDI-TOF MS is covered in UK SMI TP 40: Matrix-assisted laser desorption/ionisation - time of flight mass spectrometry (MALDI-TOF MS) test procedure. It also includes molecular methods for alternative identification and confirmation.

This document does not cover identification of *Bordetella* or *Moraxella* species. For information on identifying these genera please refer to UK SMI ID 5 – Identification of *Bordetella* species or UK SMI ID 11 – Identification of *Moraxella* species and morphologically similar organisms.

The direct identification of microorganisms from samples is beyond the scope of this document. For information related to direct identification, please refer to the other UK SMI categories.

Antimicrobial Susceptibility Testing (AST) is also beyond the scope of this document. However, for effective antibiotic stewardship, laboratories should perform AST on all clinically significant isolates, particularly in cases of poor treatment response. For further information related to AST, please refer to the other UK SMI categories.

This document addresses laboratory processes for microorganism identification and is not intended for primary healthcare guidance. For relevant information please refer to the UK SMI Syndromic documents.

UK SMIs should be used in conjunction with other relevant UK SMIs.

4 Introduction

4.1 Taxonomy and characteristics

Pseudomonas species

The genus *Pseudomonas* is a large and complex heterogeneous group of organisms belonging to the family Pseudomonadaceae in the order Pseudomonadales (1). The taxonomy of this genus is continuously being revised due to improvements in methodologies of species identification. Following DNA hybridisation studies, *Pseudomonas* species were split into 5 groups (I-V) based on rRNA homology, however groups II, III, IV and V have since been reclassified into other genera (2). The type species is *Pseudomonas aeruginosa* (1).

Pseudomonas species are Gram negative straight or slightly curved rods (3). They are non-spore forming and motile by means of one or more polar flagella. They have a very strict aerobic respiratory metabolism with oxygen but in some cases, nitrate has been used as an alternative that allows anaerobic growth (4). Most species are oxidase positive (except *Pseudomonas luteola* and *Pseudomonas oryzihabitans*) and catalase positive (5).

Pseudomonas species are split into fluorescent and non-fluorescent species. Fluorescent species (including *Pseudomonas aeruginosa, Pseudomonas fluorescens, Pseudomonas putida*) secrete pyoverdine, a fluorescent yellow-green siderophore under iron-depleted conditions (6). Certain *Pseudomonas* species may also produce additional types of siderophore, such as pyocyanin and pyochelin produced by *P. aeruginosa* (7,8).

P. aeruginosa is the glucose non-fermenting Gram negative rod most often associated with human infection. *P. aeruginosa* can grow under a variety of conditions, surviving temperatures of up to 42°C and has been isolated from a variety of samples, including human skin, throat, and stool samples as well as soil and water samples (9). *P. aeruginosa* can cause a variety of infections including pneumonia, otitis externa, lower respiratory infections in cystic fibrosis. Multi-drug resistant strains are frequently isolated and therefore accurate identification is important (10).

Infection with other *Pseudomonas* species is uncommon, occurring mostly in immunocompromised patients (5). These species include, *P. fluorescens, P. putida, P. oryzihabitans, P. luteola, Pseudomonas alcaligenes, Pseudomonas stutzeri, Pseudomonas mendocina, and Pseudomonas veronii (5).*

Other non-glucose fermenters

Burkholderia species

The genus *Burkholderia* belongs to the family Burkholderiaceae (1). Many *Burkholderia* species were reclassified from the genus *Pseudomonas.* The type species is *Burkholderia cepacia* (1). The *B. cepacia* complex (Bcc) consists of 24 closely related species most of which are opportunistic pathogens (11). All the species within the *B. cepacia* complex can cause infections in cystic fibrosis (CF) patients. *Burkholderia multivorans* and *Burkholderia cenocepacia* are the most common species associated with CF infections in the UK (12,13). Other *Burkholderia* species associated with human infection include *Burkholderia gladioli, Burkholderia pseudomallei* and *Burkholderia mallei* (14).

Burkholderia species are aerobic, non-spore forming, straight or curved Gram negative rods. Some species can reduce nitrate for anaerobic respiration (15). All species, except for *B. mallei*, are motile usually with multiple polar flagella, oxidase positive and catalase positive (14).

Stenotrophomonas maltophilia

The genus *Stenotrophomonas* has one species, *Stenotrophomonas maltophilia* that is known to cause infections in humans (14). *S. maltophilia* is a pathogen associated with infections in immunocompromised individuals (16). They are strictly aerobic, motile with one or more polar flagella, Gram negative straight or curved rods. Most strains are catalase positive and oxidase negative however some rare strains may show some oxidase positivity (16). *S. maltophilia* is often isolated from nosocomial infections.

Acinetobacter species

The genus *Acinetobacte*r belongs to the family Moraxellaceae. The type species is *Acinetobacter calcoaceticus* (1). Several similar *Acinetobacter* species have been associated with human infection. These are classified as the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex, however the species most isolated is *A. baumannii* (17).

Acinetobacter species are short, non-spore forming Gram negative rods, often becoming coccoid and appearing as diplococci (18). They may not readily decolourise on Gram staining and demonstrate variable stain retention, along with pleomorphic variations in cell size and arrangement. Many strains are encapsulated. *Acinetobacter* species are strict aerobes, oxidase negative, catalase positive, non-motile and non-fermentative (18).

Less common non-glucose fermenters

There are many other morphologically similar organisms that have occasionally been isolated from clinical specimens. These organisms are rarely isolated but should be considered. They are usually found in association with contaminated medical devices or in patients who are known to be immunocompromised. Several of these genera are emerging as nosocomial pathogens and have been linked to outbreaks in cystic fibrosis centres (19,20).

Some less common non-glucose fermenters may include, *Acidovorax facilis, Achromobacter xylosoxidans, Alcaligenes faecalis, Brevundimonas diminuta, Delftia acidovorans, Elizabethkingia mengioseptica, Comamonas terrigena, Methylobacterium organophilum, Ochrobactrum anthropic, Pandoraea apista, Ralstonia pickettii, Roseomonas gilardii, Shewanella putrefaciens, Sphingobacterium spiritivorum, Sphingomonas paucimobilis, Inquilinus limosus, Cupriavidus necator.*

5 Technical information and limitations

Identification of these species can be difficult due to similarities in characteristics. Clinicians are encouraged to ensure they are aware of any further taxonomy changes and take this into account when interpreting laboratory results. Changes in taxonomy should be considered when using commercial identification systems. All databases including MALDI-TOF MS, should be updated accordingly.

MALDI-TOF MS libraries can be biased towards clinical isolates and may not provide accurate results when identifying less commonly isolated non-glucose fermenters from non-clinical or environmental samples. Some isolates of *Pseudomonas aeruginosa* from CF patients produce alginate, which can interfere with MALDI-TOF MS identification. In this case, treatment with formic acid may be beneficial. For the MALDI-TOF MS procedure, please refer to UK SMI TP 40 – Matrix-Assisted Laser Desorption/Ionisation – Time of flight mass spectrometry.

Differentiation of species within the *B. cepacia* complex (Bcc) can be particularly problematic, even with an extended panel of biochemical tests, as they are phenotypically very similar and most commercial bacterial identification systems cannot reliably distinguish between them (21). Other organisms such as *S. maltophilia* may be misidentified as Bcc.

6 Safety considerations

The section covers specific safety considerations (22-42) related to this UK SMI, and should be read in conjunction with the general safety considerations.

B. mallei and *B. pseudomallei* are Hazard Group 3 organisms. Any suspected isolates and specimens must be handled in a containment level 3 room. All suspected *B. mallei* and *B. pseudomallei* should be handled in a safety cabinet until ruled out. If these isolates are submitted to the reference laboratory, please contact them in advance.

Laboratory-acquired infections have been reported sporadically among laboratory workers and further potential exposures have been reported (43,44). If exposed, workers should follow local protocols and may be required to start post exposure prophylaxis.

Refer to current guidance on the safe handling of all organisms documented in this UK SMI.

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

Compliance with postal and transport regulations is essential and must meet the requirements of the reference laboratory.

7 Target organisms

Please refer to Table 1 for *Pseudomonas* species and non-glucose fermenters associated with human disease.

8 Identification

Colonies on primary isolation media are presumptively identified by colonial morphology, Gram stain and oxidase activity. Further identification is determined using MALDI-TOF MS or molecular methods such as Whole Genome Sequencing (WGS). When MALDI-TOF MS is unavailable, identification can be confirmed using phenotypic tests and referral to a suitable reference or specialist laboratory.

8.1 Culture methods

Culture methods can be used to provide a presumptive identification of *Pseudomonas* species and other non-glucose fermenters prior to further identification methods such as MALDI TOF-MS.

8.1.1 Bacterial growth medium

Pseudomonas species have no specific nutritional requirements and are non-fastidious and therefore grow well on all standard laboratory media. Other non-glucose

fermenters also grow on most standard laboratory media, but some may grow best on selective media.

Primary isolation media

Isolates will grow on all standard culture media after incubation for 16-48 hours including 5% sheep blood agar in air, Chocolate blood agar in 5-10% CO_2 or MacConkey / CLED agar in air at 35-37°C (21,45). Some *Pseudomonas* species are slow growing and may require incubation for up to 72 hours (5). Some strains of *S. maltophilia* may grow best at lower temperatures (46).

For *Burkholderia* species, selective media is recommended.

Please note that visible growth should occur after 48 hours but in samples from cystic fibrosis patients, growth may be slower and therefore incubation for up to 5 days may be required.

Selective media

Selective media can be used to aid in isolation and identification of *Pseudomonas* species and other non-glucose fermenters. *Pseudomonas* selective agar can be used when isolating *P. aeruginosa.* Samples should be incubated in air at 35-37°C for 16-48 hours (5,21).

Burkholderia selective agar is commercially available and is recommended for isolation of *B. cepacia* complex and *B. pseudomallei* (21,47). For isolation using selective agar, isolates should be incubated according to manufacturer's instructions or for at least 72 hours, however growth can take as long as 5 days.

S. maltophilia has been effectively isolated using selective medium with added vancomycin, imipenem, and amphotericin B (14,21).

8.1.2 Colonial appearance

Colonial appearance varies significantly with species. *Pseudomonas* species produce colonies that are usually large and smooth with flat edges, but variants that are rough or mucoid exist (5). Colonies produced by *Burkholderia* species vary according to species and medium used. For example, *B. cepacia* complex species produce smooth colonies on blood agar but on MacConkey agar colonies can be punctate (14). For more detailed descriptions of colony morphology refer to section 8.2, Table 1.

8.2 Microscopic appearance

8.2.1 Gram stain

Please refer to UK SMI TP 39 - Staining Procedures.

All species included in this document are Gram negative straight or slightly curved rods. Some *Acinetobacter* species may not decolourise with Gram staining and have variable stain retention (18). *Acinetobacter* species can be rod-shaped or cocci (48).

For information on the microscopic appearance of individual species refer to Table 1 below.

Table 1: Microscopic and colonial appearance of *Pseudomonas* species and other non-glucose fermenters (3,5,14,15,18,48,49)

Please note that the information in this table provides general characteristics of colony appearance and can vary among different strains and culture conditions.

Species	Appearance	Additional Comments
P. aeruginosa	Rods with a single polar flagellum. Colonies are usually large and smooth with flat edges but variants that are rough or mucoid exist. Pitting is common larger colonies.	Can grow in temperatures up to 42°C.
P. putida and *P. fluorescens*	Both do not possess distinctive colony morphology.	Can grow at lower temperatures from 25 °C.
P. monteilii, P. veronii and *P. mosselii*	Motile rods. Colonies are circular and non-pigmented when grown on nutrient agar. They are also non-haemolytic on blood agar.	None
P. stutzeri	Motile rods with a singular polar flagellum Colonies are adherent and wrinkled. They can also pit or adhere to the agar and are dark brown. Colonies can become smooth and pale following inoculation.	None
P. mendocina	Colonies are smooth, non-wrinkled and flat producing a brownish yellow pigment.	Can grow at temperatures up to 41°C.

Species	Appearance	Additional Comments
P. alcaligenes and *P. pseudoalcaligenes*	Both species are motile by a singular polar flagellum. They are both non-pigmented and do not have a distinct colony morphology.	Rarely encountered. The optimum growth temperature is 35°C.
P. luteola and *P. oryzihabitans*	Rods with rounded ends and multitricous flagella. Colonies typically exhibit rough, wrinkled and adherent or, more rarely smooth colonies. They produce a non-diffusible yellow pigment.	Growth can occur at 42°C
B. cepacia complex	On MacConkey agar colonies are punctate, tenacous and appear dark pink/red. On blood agar colonies are smooth, raised and occasionally mucoid.	None
B. mallei	On nutrient agar, colonies are smooth, grey and translucent. On MacConkey agar, growth is variable.	Non-motile.
B. pseudomallei	Appear as Gram negative bacilli. Colonies are small, smooth and creamy initially. After 48 hours colonies can become wrinkled and dry.	Motile and occasionally can produce a yellow pigment.
S. maltophilia	Straight or curved rods that grow singly or in pairs. Motile with single flagella. Colonies are rough and may appear yellow or green on blood agar.	Some strains can produce slight β-haemolysis.
Acinetobacter species	Plump rods whilst growing but can become spherical during the stationary phase of growth. They often occur in pairs. Colonies are normally smooth, sometimes mucoid, usually non-pigmented.	No flagella but twitching mobility can sometimes be observed.

8.3 Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS)

MALDI-TOF MS is used as the primary method for identification of *Pseudomonas* species and other non-glucose fermenters (50). Therefore, it is important that this method is appropriately validated, manufacturer instructions carefully followed, available database updates installed and reviewed, and the use of an extraction step that can contribute to a more reliable species identification should be considered.

MALDI-TOF MS is used for the identification of *Pseudomonas* species including *P. aeruginosa* and to identify *B. pseudomallei* (51). This technique has also been utilized to aid in the identification of *Acinetobacter* species and *S. maltophilia* (52,53).

MALDI-TOF MS, however, may not identify specific species within the *B. cepacia* complex (54). In this case, further testing or referral to reference or specialist laboratories is recommended. It is important to note that when results are inconsistent, further methods should be used to ensure accurate identification of isolates.

When less common non-glucose fermenters are suspected, MALDI-TOF MS should be used for identification, when available. In cases where MALDI-TOF MS is not able to identify non-glucose fermenters, molecular methods should be used or isolates should be sent to the relevant reference or specialist laboratory (21).

8.4 Further Identification

8.4.1 Biochemical tests and commercial identification systems

Biochemical tests are no longer routinely performed in laboratories but are used in cases when MALDI-TOF MS is unavailable or when its results are inconclusive. Discrepancies in test results should be referred to the appropriate reference or specialist laboratories for further testing.

Refer to manufacturer's guidance or other relevant sources for the biochemical properties of *Pseudomonas* species and other non-glucose fermenters (5,14,48).

Please note biochemical tests are not recommended as an identification method for samples from cystic fibrosis patients (21).

Oxidase test

Please refer to UK SMI TP 26 - Oxidase Test.

Most *Pseudomonas* species are oxidase positive. *P. aeruginosa* is oxidase positive however, some strains of *P. aeruginosa* display a slow oxidase reaction (5). When coupled with colonial morphology, the results of the oxidase test can aid in providing preliminary identification for isolates (14). For oxidase test results, please see Table 2 below.

Table 2: Oxidase test results

Oxidase positive species	Oxidase negative species
P. aeruginosa	*P. luteola*
P. putida	*P. oryzihabitans*
P. fluorescens	*S. maltophilia*
P. monteilii,	*Acinetobacter* species
P. veronii	
P. mosselii	
P. stutzeri	
P. mendocina	
P. alcaligenes	
P. pseudoalcaligenes	

Commercial identification systems

Commercial identification systems may not provide definitive speciation of many of the clinically significant, non-glucose fermenters. Some kits are available for the identification of *P. aeruginosa* however for identifying other *Pseudomonas* species, commercial identification kits alone are not considered accurate (5). Commercial identification systems can be used to identify species in the *Burkholderia cepacia* complex, but they are not sufficient on their own and should be confirmed using molecular methods (55).

All identification tests should be performed from non-selective agar. Laboratories should follow manufacturer's instructions and rapid tests and kits should be verified prior to use. If confirmation of identification is required, isolates should be sent to the appropriate reference or specialist laboratory.

8.4.2 Molecular Methods

Molecular techniques have made identification of many species more rapid and precise than is possible with phenotypic techniques. However, some of these methods remain accessible to reference laboratories only and are difficult to implement for routine bacterial identification in a clinical laboratory.

Nucleic acid amplification Tests (NAAT) have been assessed for the rapid and reliable identification of *P. aeruginosa* (53,56). They have been used successfully for the rapid and reliable detection of pathogenic *Burkholderia* species – *Burkholderia mallei* and *Burkholderia pseudomallei*. The high reliability and sensitivity have also made it very

useful for screening of samples containing few organisms and potential inhibitors, which is often the case in many environmental and clinical samples (57).

Sequencing of the recA gene or WGS is recommended when organisms in the Bcc are suspected in cystic fibrosis patients, to allow accurate speciation. If unavailable in the diagnostic laboratory, isolates should be sent to the relevant reference laboratory (21).

9 Storage and Transport

For short term storage, isolates should be kept refrigerated at 4°C.

For long term storage, isolates should be frozen at -80°C or cryopreserved in line with local procedures (45).

In case of sending to laboratories for processing, ensure the specimen is placed in appropriate package and transported accordingly.

If required, save isolate on blood or nutrient agar slopes or charcoal swabs for referral to the reference or specialist laboratory.

Follow instructions provided by the reference laboratory for sending isolates. Refer to ‘Section 11: Referral to reference or specialist laboratories’ for information on sending isolates to the appropriate laboratory.

10 Reporting

10.1 Infection Specialist

Inform the medical microbiologist of presumed or confirmed *B. mallei* and *B. pseudomallei* isolates.

The medical microbiologist should be informed if the request bears relevant information to suggest infection with *B. pseudomallei* in association with:

- Foreign travel or military service.
- Laboratory, aid, or agricultural work overseas especially to Queensland (Australia), or South or Southeast Asia.

The medical microbiologist should also be informed if the presumed or confirmed glucose non-fermenting Gram negative rod is isolated from a sample taken from a normally sterile site, in accordance with local protocols.

The medical microbiologist should be informed of presumed or confirmed *Burkholderia cepacia* complex isolates from cystic fibrosis patients or pre-transplant respiratory patients, as they require confirmation with Whole Genome Sequencing.

Follow local protocols for reporting to clinicians.

10.2 Presumptive identification

If appropriate growth characteristics, colonial appearance, Gram stain of the culture and oxidase results are demonstrated.

10.3 Confirmation of identification

For confirmation and identification please see Specialist and reference microbiology: laboratory tests and services page on GOV.UK for reference laboratory user manuals and request forms.

10.4 Health Protection Team (HPT)

Refer to local agreements in devolved administrations.

10.5 UK Health Security Agency

Refer to current guidelines on Second Generation Surveillance System (SGSS) reporting (58).

10.6 Infection prevention and control team

Inform the local infection prevention and control team of presumed or confirmed isolates of *B. mallei* and *B. pseudomallei*.

11 Referral to reference or specialist laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference or specialist laboratory see user manuals and request forms

Contact appropriate reference or specialist laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England

Wales

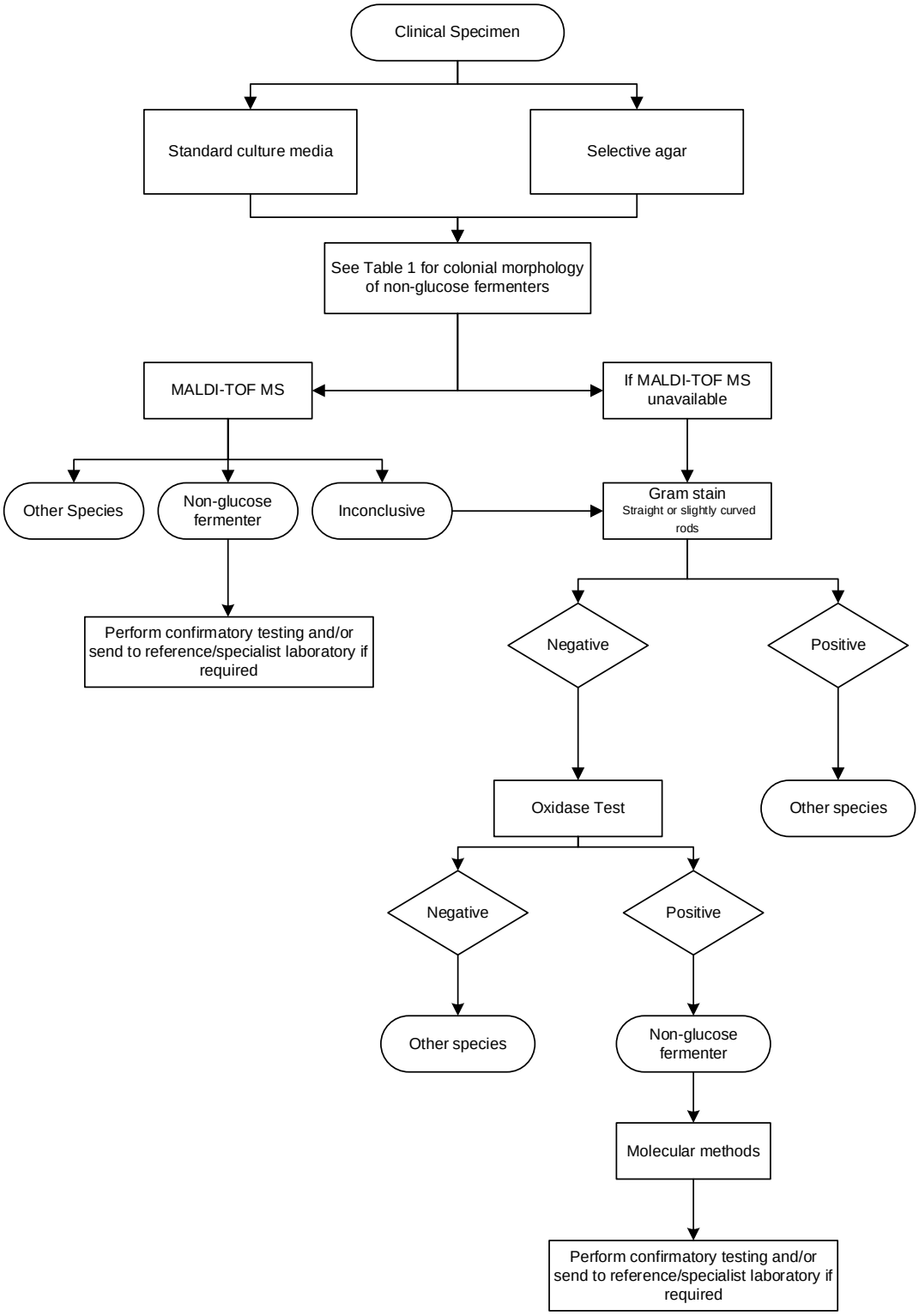
Scotland

Northern Ireland

Note: In case of sending away to laboratories for processing, ensure that specimen is placed in appropriate package and transported accordingly.

Algorithm: Identification of non-glucose fermenters

This flowchart is for guidance only.



References

An explanation of the reference assessment used is available in the scientific information section.

1. Parte AC and others. List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. International Journal of Systematic and Evolutionary Microbiology 2020: volume 70, issue 11, pages 5607-12.**++** https://doi.org/10.1099/ijsem.0.004332

2. Peix A and others. The current status on the taxonomy of Pseudomonas revisited: An update. Infection, Genetics and Evolution 2018: volume 57, pages 106-16.**++** https://doi.org/10.1016/j.meegid.2017.10.026

3. Bergey's Manual of Systematics of Archaea and Bacteria Pseudomonas; 2015. **++** https://doi.org/10.1002/9781118960608.gbm01210

4. Line L and others. Physiological levels of nitrate support anoxic growth by denitrification of Pseudomonas aeruginosa at growth rates reported in cystic fibrosis lungs and sputum. Frontiers in Microbiology 2014: volume 5.**3+** 10.3389/fmicb.2014.00554

5. Manual of Clinical Microbiology Pitout J Pseudomonas Washington, DC.: ASM Press; 2023. **++** https://www.clinmicronow.org/doi/10.1128/9781683670438.MCM.ch45

6. Kang D and others. Pyoverdine-Dependent Virulence of Pseudomonas aeruginosa Isolates From Cystic Fibrosis Patients. Frontiers in Microbiology 2019: volume 10.**3+** 10.3389/fmicb.2019.02048

7. Abdelaziz AA and others. Pseudomonas aeruginosa's greenish-blue pigment pyocyanin: its production and biological activities. Microbial Cell Factories 2023: volume 22, issue 1, pages 110.**++** 10.1186/s12934-023-02122-1

8. Noël S and others. Synthesis and biological properties of thiazole-analogues of pyochelin, a siderophore of Pseudomonas aeruginosa. Bioorganic & Medicinal Chemistry Letters 2014: volume 24, issue 1, pages 132-5.**+** https://doi.org/10.1016/j.bmcl.2013.11.054

9. Sathe N and others. Pseudomonas aeruginosa: Infections and novel approaches to treatment "Knowing the enemy" the threat of Pseudomonas aeruginosa and exploring novel approaches to treatment. Infectious Medicine 2023: volume 2, issue 3, pages 178-94.**++** https://doi.org/10.1016/j.imj.2023.05.003

10. Chan BK and others. Phage selection restores antibiotic sensitivity in MDR Pseudomonas aeruginosa. Scientific Reports 2016: volume 6, issue 1.**3++** 10.1038/srep26717

11. Depoorter E and others. Burkholderia cepacia Complex Taxon K: Where to Split? Frontiers in Microbiology 2020: volume 11.**2++** 10.3389/fmicb.2020.01594

12. Depoorter E and others. Burkholderia: an update on taxonomy and biotechnological potential as antibiotic producers. Applied Microbiology and Biotechnology 2016: volume 100, issue 12, pages 5215-29.**2++** 10.1007/s00253-016-7520-x

13. Peeters C and others. Burkholderia pseudomultivorans sp. nov., a novel Burkholderia cepacia complex species from human respiratory samples and the rhizosphere. SystApplMicrobiol 2013: volume 36, issue 7, pages 483-9.**3++** 10.1016/j.syapm.2013.06.003

14. Manual of Clinical Microbiology Burkholderia, Stenotrophomonas, Ralstonia, Cupriavidus, Pandoraea, Brevundimonas, Comamonas, Delftia, and Acidovorax Washington, DC: American Society for Microbiology; 2023. **++** https://www.clinmicronow.org/doi/10.1128/9781683670438.MCM.ch46

15. Bergey's Manual of Systematics of Archaea and Bacteria Burkholderia; 2018. pages 1-45. **++** https://doi.org/10.1002/9781118960608.gbm00935.pub2

16. Brooke Joanna S. Stenotrophomonas maltophilia: an Emerging Global Opportunistic Pathogen. Clinical Microbiology Reviews 2012: volume 25, issue 1, pages 2-41.**+** 10.1128/cmr.00019-11

17. Castanheira M and others. Global Epidemiology and Mechanisms of Resistance of Acinetobacter baumannii-calcoaceticus Complex. Clinical Infectious Diseases 2023: volume 76, issue Supplement_2, pages S166-S78.**++** 10.1093/cid/ciad109

18. Bergey's Manual of Systematics of Archaea and Bacteria Acinetobacter; 2022. pages 1-78. **++** https://doi.org/10.1002/9781118960608.gbm01203.pub2

19. Coward A and others. Structured surveillance of Achromobacter, Pandoraea and Ralstonia species from patients in England with cystic fibrosis. Journal of Cystic Fibrosis 2020: volume 19, issue 3, pages 388-93.**2++** https://doi.org/10.1016/j.jcf.2019.11.005

20. Degand N and others. Epidemic spread of Pandoraea pulmonicola in a cystic fibrosis center. BMC Infect Dis 2015: volume 15, pages 583.**3++** 10.1186/s12879-015-1327-8

21. CysticFibrosisTrust. Laboratory Standards for Processing Microbiological Samples from People with Cystic Fibrosis 2022. **++**

https://www.cysticfibrosis.org.uk/sites/default/files/2023-01/CF%20Lab%20Standards%20FINAL.pdf

22. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive 2023. pages 1-39. **++**

23. 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. 2005. pages 1-14. **++**

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

25. Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. MMWR Surveill Summ 2012: volume 61, pages 1-102.**+**

26. Department for Transport and others. Transport of infectious substances UN2814, UN2900 and UN3373 Guidance note number 17/2012 (revision 7). 2013. **++**

27. Department of Health. Health Protection Legislation (England) Guidance. pages 1-112. 2010. **++**

28. Gizzie N, Adukwu E. Evaluation of Liquid-Based Swab Transport Systems against the New Approved CLSI M40-A2 Standard. J Clin Microbiol 2016: volume 54, issue 4, pages 1152-6.**2+** 10.1128/JCM.03337-15

29. Health and Safety Executive, Advisory Committee on Dangerous Pathogens. Management and operation of microbiological containment laboratories. HSE. 2019. **++**

30. Health and Safety Executive. Risk assessment: A brief guide to controlling risks in the workplace. HSE. 2014. **++**

31. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002 (as amended). Approved Code of Practice and guidance L5 (sixth edition). HSE Books. 2013. **++**

32. Health and Safety Executive. Safe use of pneumatic air tube transport systems for pathology specimens. 2009. **++**

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

34. Home Office. Anti-terrorism, Crime and Security Act. 2001. **++**

35. Home Office. Public Health Act (Northern Ireland) 1967 Chapter 36. 1967. **++**

36. 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 1998. pages 1-37. **++**

37. UKHSA. Laboratory reporting to UKHSA: a guide for diagnostic laboratories. UKHSA 2023. pages 1-31. **++**

38. Scottish Government. Public Health (Scotland) Act. 2008. **++**

39. The Royal College of Pathologists. The retention and storage of pathological records and specimens (5th edition). pages 1-59. 2015. **++**

40. The Welsh Assembly Government. Health Protection Legislation (Wales) Guidance. 2010. **++**

41. Tyrrell KL and others. Comparison of the Copan eSwab System with an Agar Swab Transport System for Maintenance of Fastidious Anaerobic Bacterium Viability. J Clin Microbiol 2016: volume 54, issue 5, pages 1364-7.**2+** 10.1128/JCM.03246-15

42. World Health Organization. Guidance on regulations for the transport of infectious substances 2019-2020. WHO. 2019. **++**

43. BJ Currie. Laboratory exposure to Burkholderia pseudomallei--Los Angeles, California, 2003. MMWR MorbMortalWklyRep 2004: volume 53, issue 42, pages 988-90.**3+** https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5342a3.htm

44. Speiser LJ and others. Burkholderia pseudomallei Laboratory Exposure, Arizona, USA. Emerging Infectious Diseases 2023: volume 29, issue 5, pages 1061.**3+** 10.3201/eid2905.221865

45. LaBauve AE, Wargo MJ. Growth and laboratory maintenance of Pseudomonas aeruginosa. Curr Protoc Microbiol 2012: volume Chapter 6, pages Unit 6E.1.**+** 10.1002/9780471729259.mc06e01s25

46. Mahdi O and others. Laboratory culture and maintenance of Stenotrophomonas maltophilia. Curr Protoc Microbiol 2014: volume 32, pages Unit 6F.1.**+** 10.1002/9780471729259.mc06f01s32

47. Glass MB and others. Comparison of four selective media for the isolation of Burkholderia mallei and Burkholderia pseudomallei. The American journal of tropical medicine and hygiene 2009: volume 80, issue 6, pages 1023-8.**2+** https://doi.org/10.4269/ajtmh.2009.80.1023

48. Manual of Clinical Microbiology Pitout J Acinetobacter, Chryseobacterium, Moraxella, Branhamella and Other Nonfermentative Gram-Negative Rods: ASM

Press; 2023. **++**
https://www.clinmicronow.org/doi/10.1128/9781683670438.MCM.ch47

49. Lal B and others. Specimen Collection, Processing, Culture, and Biochemical Identification of Acinetobacter spp. Acinetobacter baumannii: Methods and Protocols 2019, pages 1-15.**++** 10.1007/978-1-4939-9118-1_1

50. Fernandez-Olmos A and others. MALDI-TOF MS improves routine identification of non-fermenting Gram negative isolates from cystic fibrosis patients. JCystFibros 2012: volume 11, issue 1, pages 59-62.**3+** 10.1016/j.jcf.2011.09.001

51. Watthanaworawit W and others. A multi-country study using MALDI-TOF mass spectrometry for rapid identification of Burkholderia pseudomallei. BMC Microbiology 2021: volume 21, issue 1, pages 213.**2+** 10.1186/s12866-021-02276-1

52. Li X and others. Insight into Identification of Acinetobacter Species by Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) in the Clinical Laboratory. Journal of the American Society for Mass Spectrometry 2018: volume 29, issue 7, pages 1546-53-53.**2++** 10.1007/s13361-018-1911-4

53. Bagudo AI and others. Advances in automated techniques to identify Acinetobacter calcoaceticus-Acinetobacter baumannii complex. Asian biomedicine : research, reviews and news 2020: volume 14, issue 5, pages 177-86.**++** 10.1515/abm-2020-0026

54. Wong KSK and others. Matrix-assisted laser desorption/ionization time-of-flight MS for the accurate identification of Burkholderia cepacia complex and Burkholderia gladioli in the clinical microbiology laboratory. J Med Microbiol 2020: volume 69, issue 8, pages 1105-13.**2++** 10.1099/jmm.0.001223

55. Brisse S and others. Comparative evaluation of the BD Phoenix and VITEK 2 automated instruments for identification of isolates of the Burkholderia cepacia complex. JClinMicrobiol 2002: volume 40, issue 5, pages 1743-8.**2+** https://doi.org/10.1128/jcm.40.5.1743-1748.2002

56. Boutin S and others. One time quantitative PCR detection of Pseudomonas aeruginosa to discriminate intermittent from chronic infection in cystic fibrosis. Journal of Cystic Fibrosis 2018: volume 17, issue 3, pages 348-55.**3++** 10.1016/j.jcf.2017.12.007

57. Janse I and others. Multiplex qPCR for reliable detection and differentiation of Burkholderia mallei and Burkholderia pseudomallei. BMCInfectDis 2013: volume 13, pages 86.**2+** 10.1186/1471-2334-13-86

58. UKHSA. Laboratory reporting to UKHSA: a guide for diagnostic laboratories. UKHSA 2023. pages 1-31. **++**