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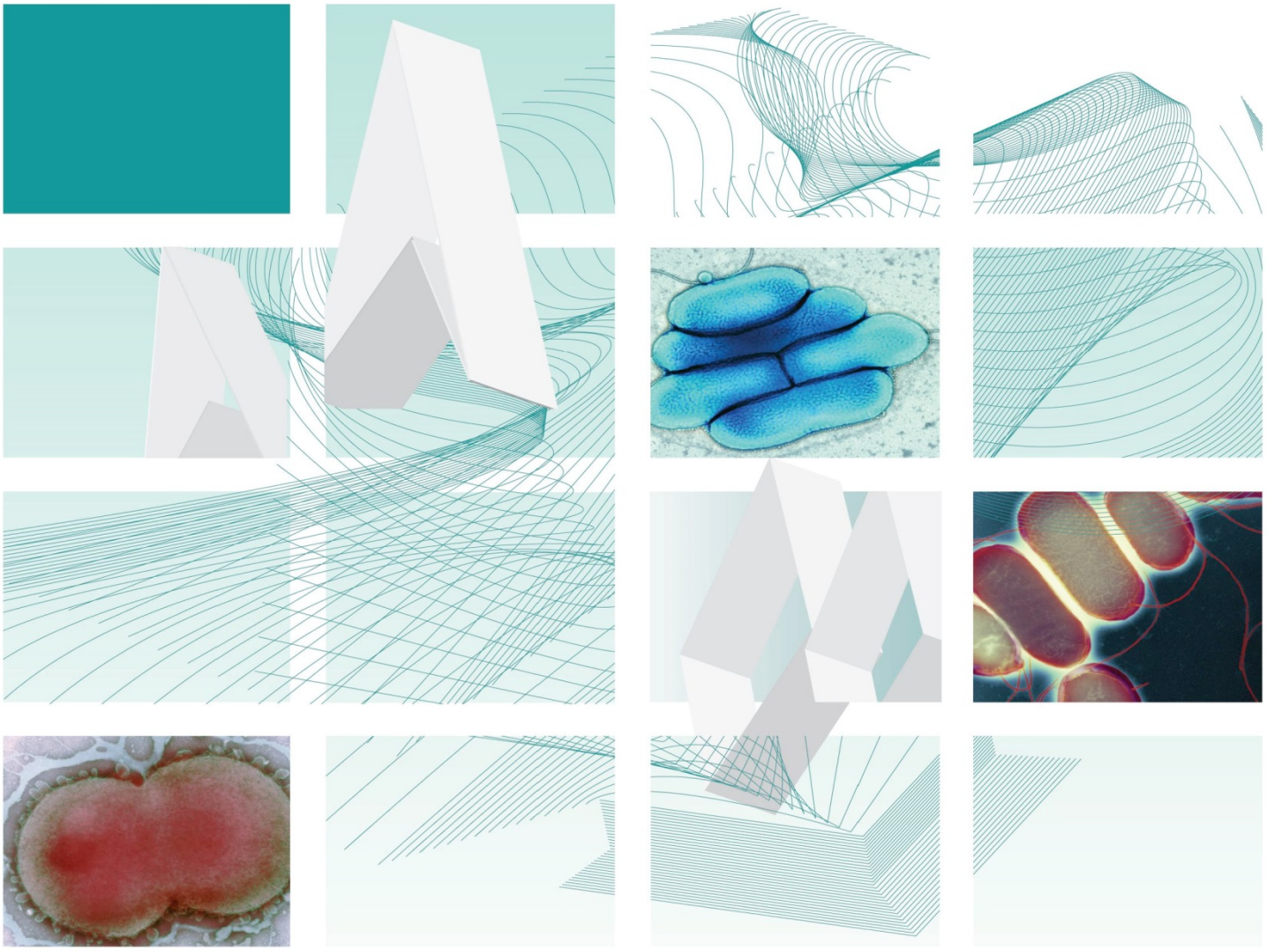
# UK Standards for Microbiology Investigations

## Identification of *Pseudomonas* species and other Non-Glucose Fermenters



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# 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).

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# Amendment Table

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

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

| Amendment No/Date. | 4/13.04.15 |
|---|---|
| Issue no. discarded. | 2.1 |
| Insert Issue no. | 3 |
| **Section(s) involved** | **Amendment** |
| Whole document. | Hyperlinks updated to gov.uk. |
| Page 2. | Updated logos added. |
| Introduction. | The taxonomy of *Pseudomonas* species and other Non-Glucose Fermenters have been updated.<br>More information has been added to the Characteristics section. The medically important species are mentioned. Other Non-Glucose Fermenters that are medically important are also mentioned and their characteristics described.<br>Section on Principles of Identification has been updated to include the MALDI-TOF. |
| Technical Information/Limitations. | Addition of information regarding Cetrimide agar Media, oxidase test and commercial identification systems. |
| Safety Considerations. | This section has been updated on the handling of *B. mallei and B. pseudomallei* and as well as laboratory acquired infections. |
| Target Organisms. | The section on the Target organisms has been updated and presented clearly. |
| Identification. | Updates have been done on 3.2, 3.3 and 3.4 to reflect standards in practice.<br>Section 3.4.3 and 3.4.4 have been updated to include MALDI-TOF MS and NAATs with references.<br>Subsection 3.5 has been updated to include the Rapid Molecular Methods. |
| Identification Flowchart. | Modification of flowchart for identification of *Pseudomonas* species and other Non-Glucose |

| | |
|---|---|
| | Fermenters have been done for easy guidance. |
| Reporting. | Subsections 5.3 have been updated to reflect the information required on reporting practice. |
| Referral. | The addresses of the reference laboratories have been updated. |
| References. | Some references updated. |

# 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.

SMIs are developed, reviewed and updated through a wide 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.

## 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

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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.

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# Scope of Document

This SMI includes the identification of *Pseudomonas* species and other non-glucose fermenters that have been associated with human infection. They are associated with a wide range of infections, predominantly of nosocomial origin. Such infections usually occur in patients with identifiable defects of local and/or systemic immunity. These bacteria can be isolated from a wide variety of environmental sources and can cause infection via contaminated medical devices or "pseudo-infection" due to their survival/growth in blood sampling tubes or laboratory media[1]. It describes their identification from selective media, and members of this diverse group of organisms from a variety of primary isolation media.

The bacteria described in this SMI are aerobic and non-sporing. They may oxidise glucose and are catalase positive. Some species are able to grow anaerobically in the presence of nitrate, and many produce water-soluble pigments[2].

For information on *Bordetella* species please see

ID 5 - Identification of *Bordetella* species

For information on *Moraxella* species please see

ID 11 - Identification of *Moraxella* species and Morphologically Similar Organisms

This SMI should be used in conjunction with other SMIs.

# Introduction

## Taxonomy[1,3,4]

The genus *Pseudomonas* is a large and complex heterogeneous group of organisms belonging to the family Pseudomonadaceae containing 211 validly described species but 56 of which have been reclassified to other genera. They are constantly undergoing continuous taxonomic revision due to improvements in methodologies of species identification. Organisms previously classified within the genus *Pseudomonas* (rRNA homology groups I-V) are now divided among the genera *Pseudomonas, Burkholderia, Ralstonia, Comamonas, Acidovorax, Delftia, Hyrodenophaga, Brevundimonas*, *Stenotrophomonas* and *Xanthomonas*. Many identified strains have no designated species. Commercial identification systems do not provide definitive speciation of many of the clinically significant, glucose non-fermenting Gram negative bacilli. In clinical situations where precise identification is important for determining optimal therapy, patient prognosis, and appropriate infection control interventions (eg if querying the first isolation of a member of the *Burkholderia cepacia* complex in a respiratory sample from a patient with cystic fibrosis), referral of such an isolate to a Reference Laboratory is usually appropriate.

## Characteristics

### *Pseudomonas* species[3]

*Pseudomonas* species are aerobic, non-spore forming, Gram negative rods which are straight or slightly curved and are 0.5 – 1.0µm by 1.5 – 5.0µm. They are 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. Most species are oxidase positive (except *P. luteola* and

*P. oryzihabitans*) and catalase positive. Other characteristics that tend to be associated with *Pseudomonas* species (with some exceptions) include secretion of pyoverdine, a fluorescent yellow-green siderophore under iron-limiting conditions[5]. Certain *Pseudomonas* species may also produce additional types of siderophore, such as pyocyanin by *Pseudomonas aeruginosa* and thioquinolobactin by *Pseudomonas fluorescens*[6].

They grow well on standard broth and solid media such as blood agar, chocolate agar, and MacConkey agar, which are recommended to isolate *Pseudomonas* species from clinical specimens. Selective agar containing inhibitors such as cetrimide can also be used for isolation and presumptive identification. *Pseudomonas* colonies may be nearly colourless, but white, off-white, cream, and yellow colony pigmentation is common. Fluorescent colonies can be readily observed under ultraviolet light.

The type species is *Pseudomonas aeruginosa*.

**The medically important species are as follows;**

***Pseudomonas aeruginosa*[1]**

*P. aeruginosa* is the glucose non-fermenting Gram negative rod most often associated with human infection. It has the characteristic grape-like smell of aminoacetophenone. It is a strict aerobe with a growth temperature range of 5-42°C. Most other pseudomonads will not grow at 42°C (with certain exceptions, notably *Burkholderia pseudomallei*). The characteristic blue-green appearance of colonised/infected pus or of an organism culture is due to the mixture of pyocyanin (blue) and pyoverdin (fluorescein, yellow). Production of blue-green pigment is indicative of *P. aeruginosa*[1]. Some strains produce other pigments, such as pyorubin (red) or pyomelanin (brown). Almost all strains are motile by means of a single polar flagellum.

*P. aeruginosa* can produce at least six colonial types after aerobic incubation on nutrient agar for 24hr at 37°C. The most common, type 1, is that of colonies which are large, low, oval, convex and rough, sometimes surrounded by serrated growth. Colonial variation from one type to another does not necessarily indicate the presence of more than one strain. Many strains exhibit metallic iridescence with colonial lysis. This resembles lysis by bacteriophage, but is not associated with phage activity.

Colonies isolated on *Pseudomonas* selective or blood agar may be presumptively identified by a positive oxidase reaction and characteristic pigment production as '*P. aeruginosa'*. However, some strains of *P. aeruginosa*, particularly the mucoid ones, may not produce pyocyanin, as well as displaying a slow oxidase reaction and may therefore require further tests to confirm identification. Colonies isolated on other selective agars (such as Bcc) may be identified by colonial morphology and a commercial identification system. Other species from blood or selective media and strains of *P. aeruginosa* and *B. cepacia* complex requiring further characterisation should be identified by a commercial identification system and/or referral to a Reference Laboratory. It should be noted that isolates from cystic fibrosis patients can be atypical/stressed and should be incubated at 30°C or room temperature for 48hrs so that their phenotypic features may reliably be expressed.

**Other *Pseudomonas* species[3]**

Infection with such organisms is relatively uncommon. When it does occur, it is usually in a patient with compromised immune defence(s) or is associated with a contaminated medical device[7]. However, accurate recognition of the infecting

organism can be important as antimicrobial susceptibility varies widely among these organisms. Pseudo-infections have also been reported.

*Pseudomonas putida* and *Pseudomonas fluorescens* are members of the fluorescent group of pseudomonads. Unlike *P. aeruginosa,* they are unable to grow at 42°C and do not produce pyocyanin. *P. putida* can be distinguished from these other 2 species by its inability to liquefy gelatine[7].

*Pseudomonas monteilii*, *Pseudomonas otitidis* and *Pseudomonas mosselii* produce circular and non-pigmented colonies when grown on nutrient agar. They are also both non-haemolytic on blood agar[8,9].

*Pseudomonas stutzeri* produces smooth, intermediate and rough colonies (sometimes yellow pigmented) when grown on nutrient agar[10]. They can also pit or adhere to the agar and are buff to brown. The latter can resemble colonies of *Burkholderia pseudomallei* or *Bacillus* species.

*Pseudomonas mendocina* produces smooth, non-wrinkled and flat colonies producing a brownish yellow pigment.

*Pseudomonas alcaligenes* and *Pseudomonas pseudoalcaligenes* are rarely encountered in clinical specimens. They are both non-pigmented and do not have a distinct colony morphology. They are biochemically inert when compared with other pseudomonads.

*Pseudomonas luteola* and *P. oryzihabitans* typically exhibit rough, wrinkled, adherent colonies or, more rarely, smooth colonies. They can both be distinguished from other pseudomonads by their negative oxidase reaction and production of non-diffusible yellow pigment.

Primary culture for *Pseudomonas* species should be performed on blood agar and/or *Pseudomonas* selective agar. Colonial appearance of *Pseudomonas* species is described in Section 3.3. Clinically significant isolates may need to be referred to the Reference Laboratory for further characterisation.

**Morphologically Similar Organisms**

***Burkholderia* species**

There are currently 82 validly published species of the genus *Burkholderia* but in recent years, three species have been reclassified to genera *Pandoraea* and *Ralstonia*[11].

**The medically important *Burkholderia* species are;**

***Burkholderia cepacia* complex**

Recent research has resulted in a number of changes to the taxonomy of *Burkholderia cepacia* complex (Bcc). The Bcc currently comprises 17 species which exhibit a high degree of 16S rRNA (98–100%) and recA (94–95%) gene sequence similarity, and moderate levels of DNA–DNA hybridization (30–50%)[12]. They include *B. cepacia, B. multivorans, B. stabilis, B. vietnamiensis, B. ambifaria, B. athina, B. pyrrocinia, B. latens, B. diffusa, B. arboris, B. seminalis, B. cenocepacia, B. contaminans, B. dolosa, B. lata, B. ubonensis* and *B.metallica*[11,13]. A new specie within the Bcc has been proposed with *B. multivorans* as its nearest phylogenetic neighbour. The species is *B. pseudomultivorans* sp. nov.[14]. Certain genomovars/species have been more closely associated with hospital outbreaks and clinical disease in susceptible patients (eg *B. cepacia* genomovar III and outbreaks of fulminant pneumonitis in CF units[12]).

Some Bcc strains may be isolated from contaminated medical devices such as blood gas analysers, nebuliser equipment or disinfectants[15-17].

Bcc organisms are slender motile rods. They grow aerobically but prefer temperatures of 25-35°C for optimal growth. Most strains will grow at 41°C, but not at 42°C, and no strains grow at 4°C[1].

Primary culture for Bcc should be performed on a *B. cepacia* selective agar. Examples include *Burkholderia cepacia* selective agar (BCSA), *Burkholderia cepacia* agar (BCA) (formerly known as *Pseudomonas cepacia* agar or PCA), and Oxidation-Fermentation Polymyxin Bacitracin Lactose agar (OFPBL). Recent evaluations suggest that BCSA is more selective and grows Bcc colonies more rapidly than the others[18,19]. All contain antibiotics to improve selectivity. Media should be incubated at 35–37°C for 2 days. Some strains may appear only if the plates are further incubated at 30°C for up to 5 days. Colonial appearances vary according to the medium employed. It is important that presumptive isolates of *B. cepacia* are identified as rapidly as possible to assist in patient management. Bcc can be nitrate negative and ONPG positive. The oxidase reaction of *B. cepacia* varies in strength. Isolates may become non-viable when stored at ambient temperature or 4°C for several days. Presumptive identification of Bcc from CF patients should first be carried out with a commercial identification system, although these remain unreliable for confirmation of Bcc[20,21]. All first time isolates suspected to be Bcc should therefore be referred to a Reference Laboratory for confirmation of identity, species and genomovar.

## Other *Burkholderia* species

***Burkholderia mallei*** is a Hazard Group 3 pathogen. *B. mallei* is a small non-motile, usually oxidase negative, Gram negative bacillus. The bacterial cell may be straight or slightly curved with rounded ends and wavy sides. The bacilli may be arranged singly, in pairs end to end, in parallel bundles or palisades. These organisms are rare and not identifiable with commercial kits.

***Burkholderia pseudomallei*** is also a Hazard Group 3 pathogen. However, in contrast, it is a Gram negative, oxidase positive, motile bacillus. Collectively they may appear as long bundles, but actually these represent chains of densely packed organisms. In clinical material the staining may be irregular and bipolar staining may be seen. *B. pseudomallei* is nitrate positive and ONPG negative. It is the aetiological agent of idosis.

Definitive diagnosis of melioidosis is by positive culture of *B. pseudomallei*, but the results may be obtained too late to influence clinical management. On nutrient agar, rough corrugated colonies resembling *P. stutzeri* may be produced and cultures often have a pearly sheen, although there is considerable colonial variation. Some strains may produce dry and wrinkled colonies whereas others may be frankly mucoid. Usually, the colonies are not coloured, but occasional strains may produce a yellow pigment. It grows well at 42°C. Pictures of the colonies of *P. stutzeri* and B*. pseudomallei* that may have similar colonial appearances can be seen on the PHE website. Suspect colonies should be referred to the Reference Laboratory.

Isolates of *B. pseudomallei* are constitutively resistant to polymyxin and aminoglycosides, but susceptible to co-amoxiclav, Trimethoprim/sulfamethoxazole (TMP/SMX) plus doxycycline[22]. Melioidosis may also be diagnosed serologically, although results can be difficult to interpret due to elevated background levels of antibody in endemic areas. *B. pseudomallei* should be considered in patients with

pneumonia, septicaemia or abscesses who have a history of travel to South East Asia or Northern Australia, particularly those with underlying conditions such as diabetes mellitus.

***Burkholderia gladioli*** grow readily on media containing polymyxin. Unlike Bcc they are oxidase negative and do not oxidise maltose and lactose. *B. gladioli* are occasionally isolated from the respiratory tract of patients with CF but, unlike Bcc, its clinical significance in these patients remains uncertain. Molecular methods such as PCR are required to confirm its identity[23].

Other *Burkholderia* species that have been known to cause infections in humans are *B. caledonica* and *B. fungorum*.

### *Stenotrophomonas maltophilia*[24]

*S. maltophilia* is also a commonly isolated glucose non-fermenter in clinical laboratories. It may cause a wide range of infections (such as intravascular line-associated bacteraemias and nosocomial pneumonia) in susceptible patients, notably those with an underlying haematological malignancy. However, in other settings isolates often represent superficial colonisation only. *S. maltophilia* is oxidase negative and motile. It can appear as straight or slightly curved non-sporulating rods. Rare strains may be slow to show oxidase positivity. Colonies may appear yellow or green on blood agar. Resistance to imipenem in vitro is a useful indicator to suspect *S. maltophilia*. Some strains may produce slight beta-haemolysis. Although growth has been reported to occur between 5°C and 40°C, it is optimal at 35°C. Most commercial identification kits are able to identify the bacterium.

They have been isolated from a wide range of nosocomial sources[24].

### *Acinetobacter* species

Based on DNA-DNA hybridization studies, there are now 32 validly published *Acinetobacte*r species[25]. Fifteen of these have been associated with human infections, namely *A. calcoaceticus, A. baumannii, A. beijerinckii, A. bereziniae, A. gyllenbergii, A. haemolyticus, A. junii, A. johnsonii, A. nosocomialis, A. parvus, A. pittii A. lwoffi*, *A. schindleri, A. ursingii,* and *A. radioresistens*. In clinical practice, *A. baumannii* is most frequently isolated, notably from intensive care units, and is often extensively antimicrobial resistant. Other more commonly isolated species are *A. calcoaceticus, A. lwoffi, A. johnsonii* and *A. haemolyticus*. Interpreting the significance of *Acinetobacter* isolates from clinical specimens is often difficult, because of their wide distribution in nature and their ability to colonise healthy or damaged tissue - they have been isolated as contaminants in patient samples, probably due to environmental sources or cross contamination between patients and hospital staff[26,27].

*Acinetobacter* species are short, plump Gram negative rods/coccobacilli, typically 1.0-1.5 by 1.5-2.5µm, often becoming coccoid and appearing as diplococci. 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. Colonies are normally smooth, sometimes mucoid, pale yellow to greyish-white and some environmental strains may produce a diffusible brown pigment. Some clinical isolates, particularly *Acinetobacter haemolyticus,* may be haemolytic on blood agar. Colony size is similar to that of the *Enterobacteriaceae* from which they need to be distinguished. *A. lwoffi* and some other species are 0.5µm or

less at 24-48hr. Most strains have an optimum growth temperature of 30-35°C and grow well at 37°C although some are unable to grow at 37°C[28].

*Acinetobacter* species are strict aerobes, oxidase negative, catalase positive, non-motile and non-fermentative. Most commercial identification kits can distinguish *Acinetobacter* species from other non-fermenters and *Enterobacteriaceae.* However, phenotypic identification methods for individual *Acinetobacter* species can be unreliable – hence clinically or epidemiologically relevant isolates should be referred to a Reference laboratory.

They have been isolated from tracheal aspirate, blood culture, urine, cerebrospinal fluid (CSF), central venous line, wound swab and faeces[27,28].

**Other less common morphologically similar organisms**

There are many other morphologically similar organisms that have occasionally been isolated from clinical specimens. They are usually found in association with contaminated medical devices or in patients who are known to be immuno-compromised. Some may occasionally be isolated from the respiratory tract of patients with chronic lung infections such as cystic fibrosis or bronchiectasis. It may be difficult to confirm the identity of some of these organisms with commercial identification kits, and molecular identification may be needed to confirm the organism's identity[29,30]. In such cases it may be appropriate to refer these isolates to a Reference Laboratory.

The occasionally isolated morphologically similar organisms include:

***Acidovorax* species**

There are currently 15 validly published species and one subspecies of the genus *Acidovorax*[31]. The species that are associated with infection in humans are *Acidovorax delafieldii, Acidovorax facilis* and *Acidovorax temperans* all previously classified as *Pseudomonas* species[32].

Cells are Gram negative straight to slightly curved rods, 0.2 - 0.7 by 1.0 - 5.0µm. They occur singly or in short chains and are motile by means of a single polar flagellum. They are aerobic and optimum growth temperature is 35-37°C. On blood agar, colonies exhibit a creamy yellow colour, rough and raised appearance and are approximately 1.5-2.5mm in diameter.

Some strains grow on Christensen urea agar, but lack urease according to API 20NE tests. They appear non-pigmented on nutrient agar. *Acidovorax facilis* and several *Acidovorax delafieldii* strains are capable of lithoautotrophic growth by using hydrogen as an energy source. Oxidative carbohydrate metabolism occurs with oxygen as the terminal electron acceptor; alternatively, some strains of *Acidovorax delafieldii* and *Acidovorax temperans* are capable of heterotrophic denitrification of nitrate. Good growth is obtained on media containing organic acids, amino acids, or peptone and only a limited number of sugars are used for growth. They are oxidase positive and urease activity varies among strains[32].

*Acidovorax* species have been isolated from clinical specimens - urine, nasopharynx, central venous catheter, wound secretion, open fracture and sputum[32].

The type species is *Acidovorax facilis*.

### *Achromobacter* species

There are now 11 validly published *Achromobacter* species. Eight of these have been known to cause infections in humans namely; *A. insolitus, A. mucicolens, A. animicus, A. pulmonis, A. piechaudii, A. spanius, A. spiritinus* and *A. xylosoxidans*[33,34].

Cells are gram negative straight rods that occur as singly or in pairs and are motile by using 1–20 peritrichous flagella. They are strictly aerobic and grow at 35-37°C. Most species do not grow well at 42°C apart from *A. xylosoxidans, A. insolitus* and *A. pulmonis*. On trypticase soy agar, colonies are slightly convex, translucent and non-pigmented, with smooth margins and about 0.5–2mm in diameter after 48hr incubation. They are catalase and oxidase positive. They are also positive for nitrate reduction tests, and do not assimilate maltose[35].

*Achromobacter* species has occasionally been isolated from respiratory secretions of patients with CF and has also caused sepsis in other patients who are immuno-compromised[35-37].

### *Alcaligenes* species

There are currently two validly published species namely; *Alcaligenes faecalis* and *Alcaligenes eutrophus*[38].

*Alcaligenes faecalis* is the type species and is associated with human infections[39]. It has three subspecies but only *Alcaligenes faecalis* subsp. *faecalis* has been isolated from humans. Cells are Gram negative rods, coccobacilli or cocci occurring singly. Their optimal growth temperature is 20-37°C. Colonies have a thin, spreading irregular edge with no pigmentation. They are catalase positive, oxidase positive and motile by peritrichous flagella.

They have been isolated from blood, urine, tonsils, pus and faeces[39].

### *Brevundimonas* species

There are currently 24 validly published species[40]. Species known to have caused infections in humans are *B. faecalis, B. vancanneytii, B. vesicularis* and *B. diminuta*.

Cells are Gram negative and appear as straight slender non-sporing rods on Gram stain. They are aerobic and their optimal growth temperature is 30-37°C. They are also motile by a single polar flagellum. They are oxidase positive and give variable results on catalase although usually positive.

*Brevundimonas vesicularis* and *Brevundimonas diminuta* grow slowly on ordinary nutrient media. Colonies of *B. diminuta* on MacConkey agar are chalk white, whereas colonies of *B. vesicularis* are characterised by an orange intracellular pigment[41].

They have been isolated from skin lesion, faeces and blood[42].

### *Delftia* species

There are currently four validly published species and two of which are known to cause infections in humans - *Delftia acidovorans* and *Delftia tsuruhatensis*[43]. Cells are Gram negative straight to slightly curved rods, 04-0.8 x 2-5-4.1µm (occasionally up to 7µm), which occur singly or in pairs. They are motile by means of polar or bipolar tufts of one to five flagella. They are oxidase and catalase positive. Endospores are not produced, and no fluorescent pigments are produced. They are strictly aerobic and grow well on media containing organic acids, amino acids, peptone and carbohydrates (but not glucose)[44]. On nutrient agar, there is no pigment production.

*Delftia acidovorans (*formerly *Comamonas acidovorans*) characteristically produces an orange indole reaction due to anthranilic acid rather than indole production from tryptophan.

The type species is *Delftia acidovorans*.

### *Elizabethkingia* species

There are currently three validly published species of *Elizabethkingia*[45].

*Elizabethkingia* (formerly *Chryseobacterium) meningoseptica*, is the species of *Elizabethkingia* most often associated with serious infection. Although rare, it is important to identify the organism as outbreaks may occur in nurseries and the mortality rate has been described as high as 50 %[46,47]. Cells are Gram-negative, non-motile, non-spore-forming rods (0.5 x1.0–2.5µm). Good growth is observed on Trypticase soy agar and blood agar at 28–37°C, but no growth is observed at 42°C. Colonies are white–yellow, non-pigmented, semi-translucent, circular and shiny with entire edges. Catalase and oxidase activities are positive. *E. meningoseptica* is non-motile and hydrolyses aesculin and gelatin, is positive for the o-nitrophenyl-b-galactopyranoside (ONPG) test, and produces indole. However, the indole reaction is described as only weakly positive after 48hr incubation at 30°C, and a more robust reaction is observed with inoculation to Brain Heart Infusion broth rather than tryptophan broth.

### *Comamonas* species

There are currently 16 validly published species of *Comamonas*[48]. *Comamonas terrigena* and *Comamonas testosteroni* are the only species that has been associated with human infections.

Cells are Gram negative straight or slightly curved, rod shaped, and 0.5 - 1 by 1 – 4µm. The cells occur singly or in pairs and are motile by means of a tuft of polar flagella. Endospores are not produced. They are oxidase and catalase positive. They are strictly aerobic, non-fermentative, and chemoorganotrophic[49]. On blood agar, colonies appear pink-pigmented with a mucoid and bulgy surface. They show no haemolysis on both blood and chocolate agar[50].

Good growth is obtained on media containing organic acids, amino acids, or peptone. No fluorescent pigments are produced. Carbohydrates are rarely attacked.

It has been isolated from soil and human blood, intravenous lines, urine, cerebrospinal fluid, appendix and abdominal abscess[51].

*C. terrigena* is the type species.

### *Methylobacterium* species

There are currently 49 validly published species of *Methylobacterium* and infections reported from normally sterile sites were from immuno-compromised patients[52,53].

*Methylobacterium* species colonies grow slowly on blood agar and are dry and appear pink or coral in incandescent light[54]. Optimum growth occurs at 25-30°C. The organism is oxidase positive and motile, but both of these characteristics may be weak. *Methylobacterium* species are Gram negative but may stain poorly or show variable results and may be confused with *Rhodococcus or Roseomonas* species. It has a characteristic microscopic appearance because individual cells contain large, non-staining vacuoles.

They have been isolated from blood, dialysate, lymph node, bone marrow, skin and synovium[53].

### *Ochrobactrum* species

There are currently 17 validly published species of the genus *Ochrobactrum*[55]. Five species have been known to cause infections in humans namely; *Ochrobactrum anthropi* , *Ochrobactrum haematophilum, Ochrobactrum intermedium, Ochrobactrum pseudintermedium* and *Ochrobactrum pseudogrignonense*.

Cells are Gram negative non-sporing rods with parallel sides and rounded ends; usually arranged singly. They are strict aerobes and motile by peritrichous flagella. The optimal growth temperature is 20-37°C. Colonies are 1mm in diameter on blood agar after 24 hours incubation and appear circular, low convex, smooth, and shining[56]. Mucoid colonies may be produced on some media. Colonies exhibit brown melanin-like pigmentation on tyrosine agar. They are also oxidase and catalase positive.

*Ochrobactrum anthropi* is the type species.

They have been isolated from blood, peritoneal fluid and catheters[56].

### *Oligella* species[57]

There are currently only two species in this genus; *O. ureolytica* (previously known as CDC group IVe) and *O. urethralis*[58].

For more information on the two species isolated from humans, please see

ID 11 - Identification of *Moraxella* species and Morphologically Similar Organisms.

They are small rods, mostly not exceeding 1µm and often occurring in pairs. The cells lack the plumpness of moraxellas. They are non-capsulated, non-spore-forming and mostly non-motile, but some strains of *O. ureolytica* are peritrichously flagellated. They are aerobic and grow on nutrient agar but with the addition of yeast, autolysate, serum or blood. Colonies on blood agar develop rather slowly and more overtly white than all recognised species of *Moraxella*. No pigments or odour are produced. They are also non-haemolytic.

They are oxidase positive and usually catalase positive. They do not ferment or oxidise carbohydrates. They are mainly isolated from the genitourinary tract of humans.

### *Pandoraea* species

There are currently nine validly published species and five of which are known to cause infections in humans[59]. They include; *Pandoraea apista, Pandoraea norimbergensis* (formerly *Burkholderia norimbergensis*), *Pandoraea pulmonicola, Pandoraea pnomenusa* and *Pandoraea sputorum*[60]. Their closest related genera are *Burkholderia* and *Ralstonia* but can be differentiated by their specific 16S rRNA restriction profile[41].

Cells are Gram negative, non-sporing, straight rods of 0.5-0.7 by 1.5-4.0µm. They occur singly and are motile by means of a single polar flagellum. They are catalase positive and negative for nitrate reduction, DNase and indole production. They give variable results on oxidase activity. They grow at 30 and 37°C. Colonies are white, circular and convex with clear margins.

They have been isolated from human clinical samples – specimens from the upper airways, lung tissue, urine, wound, sputum (mostly cystic fibrosis patients), blood from

patients with chronic obstructive pulmonary disease or CGD and the environment. They have also been isolated from powdered milk and sludge.

The type species is *Pandoraea apista*.

### *Psychrobacter* species[61,62]

There are currently 34 valid species and six of which have been isolated from humans[63]. They are as follows; *P. arenosus, P. immobilis, P. faecalis, P. phenylpyruvicus, P. pulmonis* and *P. sanguinis.*

For more information on the six species isolated from humans, please see

ID 11 - Identification of *Moraxella* species and Morphologically Similar Organisms.

*Psychrobacter* cells are non-motile, Gram negative coccobacilli which are often found as diploforms, measuring 0.9-1.3 x 1.5-3.8µm. The organisms are oxidase positive, with a strictly oxidative metabolism and demonstrate a moderate halotolerance.

Unlike the moraxellae, many *Psychrobacter* species are able to form acid aerobically from glucose and several other sugars. They are able to grow at 5°C and have optimal temperature near 25°C. They are generally unable to grow at 35 to 37°C although some strains have an optimal growth temperature of 35 to 37°C. Colonies on heart infusion agar are cream-coloured, unpigmented, smooth and opaque with a buttery consistency. Some *Psychrobacter* species isolates can be occasionally pale pink, possibly owing to accumulated cytochrome proteins. They are also positive catalase and tributyrin esterase, and susceptible to colistin, but negative for alkaline phosphatase, trypsin, pyrrolidonyl aminopeptidase, production of indole, β-galactosidase (ONPG), gelatin, aesculin hydrolase and arginine dihydrolase, and for growth at 42°C.

Their habitats range from glacier mud in Antarctica to human tissues, making them interesting organisms for the medical profession as well as microbiological and environmental research.

### *Ralstonia* species

There are currently five validly published species and three of which are known to cause infections in humans namely; *Ralstonia insidiosa, Ralstonia mannitolilytica* and *Ralstonia pickettii*. Other species that used to be within this genus have been reclassified to the genus *Cupriavidus*[64].

Cells are Gram negative, slender, straight non-sporing rods. They are aerobic and motile. The optimal growth temperature is 30-37°C. They grow slowly on primary isolation media, requiring about 72hr of incubation before colonies are visible. They resemble Bcc on selective agar and can be difficult to distinguish from it biochemically[65]. They are oxidase positive and catalase positive (except that some catalase negative strains of *Ralstonia pickettii* have been reported)[41].

### *Roseomonas* species

The genus comprises 17 validly published species of which six have been reported to cause infection namely; *Roseomonas cervicalis, Roseomonas fauriae, Roseomonas gilardii* (has two subspecies; *gilardii and rosea*), *Roseomonas mucosa, Roseomonas ludipueritiae, Roseomonas rosea*[66].

Members of the genus are gram negative, non-fermentative, plump coccoid rods, appearing in pairs or short chains or may be mainly cocci with occasional rods[67]. They grow on 5% sheep blood agar, heart infusion agar with 5% rabbit blood, chocolate

agar, BCYE agar, Trypticase soy agar, and almost always (91%) on MacConkey agar but do not grow in media containing 6% or more NaCl. The optimum growth temperature is 25-37°C. Growth on blood agar is pinpoint, pale pink, shiny, raised, and often mucoid after 2-3 days' incubation at 35-37°C[68,69]. *Roseomonas* species produce red-pink pigment[70].

They are weakly oxidase positive or oxidase negative, catalase positive and urease positive.

*Roseomonas* species are pathogenic for humans, causing bacteraemia and wound, urinary tract, and other infections. These species have been isolated from the aquatic environment and various clinical samples, such as blood and wound.

The type species is *Roseomonas gilardii*.

***Shewanella* species**

There are currently 62 validly published species and only one of which is known to cause infections in humans namely; *Shewanella putrefaciens*[71].

Cells are Gram negative straight or curved rods. They are motile by polar flagella. They are oxidase and catalase positive. The optimum growth temperature is 25-35°C Colonies are distinctive smelling and produce an orange-tan pigment on blood agar[72].

***Sphingobacterium* species**

There are currently 27 validly published species and only two of which are known to cause infections in humans namely; *Sphingobacterium multivorum* and *Sphingobacterium spiritivorum* (previously classified as *Flavobacterium* species)[73]. These two species are isolated most frequently from clinical specimens (sputum, blood) and have been associated with bacteraemia, peritonitis, and chronic respiratory infection in patients with severe underlying conditions[74].

Cells are Gram negative, non-sporing rods and non-motile. They are aerobic. They grow at 5°C and 37°C but not at 42°C. They grow well on blood agar, Muller- Hinton agar, MacConkey agar, *Burkholderia cepacia* selective agar (BCSA) and Sabouraud agar at 37°C for up to 72hr. On nutrient agar after two days incubation, colonies are circular, entire, low convex, smooth and opaque and a yellow or creamy white, non-fluorescent pigment is produced. Catalase, oxidase, urease, extracellular deoxyribonuclease and phosphatase (alkaline and acid) reactions are produced. Neither indole nor gelatinase are produced.

The type species is *Sphingobacterium spiritivorum*.

## Principles of Identification

Colonies on primary isolation media are presumptively identified by colonial morphology, Gram stain, oxidase activity and pigment production. The oxidase reaction is an important discriminatory test. Oxidase positive, glucose non-fermenting, Gram negative bacilli such as *Pseudomonas aeruginosa* may be termed as "pseudomonads". Additional identification may be made using a commercial identification kit.

Further identification is determined by further phenotypic tests and/or referral to a suitable Reference Laboratory. All identification tests are ideally performed from non-selective agar.

# Technical Information/Limitations

### Commercial Identification Systems

Basic commercial ID systems may be limited in their ability to identify accurately glucose non–fermenters and these organisms can be very time consuming to identify by phenotypic tests. Differentiation of species within the *B. cepacia* complex 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[3]. Other organisms such as *S. maltophilia* may be misidentified as Bcc. Commercial kits may also misidentify *Brucella* species as *Psychrobacter phenylpyruvicus*[75].

All identification tests should ideally be performed from non-selective agar. It is essential that laboratories follow the manufacturers' instructions when using commercial identification tests. Careful consideration should be given to isolates that give an unusual identification. If confirmation of identification is required, isolates should be sent to the Reference Laboratory.

### Cetrimide Agar Media

*P. aeruginosa* can lose its fluorescence under UV if the cultures are left at room temperature for a short time. Fluorescence reappears when plates are re-incubated[76].

### Oxidase Test

*P. aeruginosa* is oxidase positive however, some strains of *P. aeruginosa*, particularly the mucoid ones display a slow oxidase reaction and may therefore require further tests to confirm identification[1].

# 1 Safety Considerations[77-93]

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

*B. mallei* cause the disease called glanders while *B. pseudomallei* cause the infectious disease "Melioidosis", also called Whitmore's disease. Laboratory-acquired infections have been reported sporadically among laboratory workers - there have been twenty cases of infection with seven deaths, reported up to 1976, 3 cases of infections in 1992 and also a case in 2000[94,95]. These were mostly due to mouth pipetting, accident using equipment and skin contact exposure.

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

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[85].

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

Compliance with postal and transport regulations is essential.

# 2 Target Organisms[7,9,13-17,24,28,32,35-37,39,42,46,47,50,51,53,54,56,65,68,72,74,96-99]

***Pseudomonas* species commonly isolated in the clinical laboratory** – ***P. aeruginosa****, P. putida, P. fluorescens, P. monteilii, P. otitidis, P. mosselii, P. stutzeri, P. mendocina, P. alcaligenes, P. pseudoalcaligenes, P. luteola, P. oryzihabitans*

**Other morphologically similar organisms that have occasionally been isolated in clinical specimens – *Acinetobacter* species*, Burkholderia cepacia* complex*, Stenotrophomonas maltophilia,*** *Burkholderia caledonica, Burkholderia fungorum, Burkholderia mallei, Burkholderia pseudomallei, Burkholderia gladioli, Delftia acidovorans, Delftia tsuruhatensis, Sphingobacterium multivorum, Sphingobacterium mizutaii, Sphingobacterium spiritivorum, Psychrobacter* species*, Oligella* species*, Elizabethkingia meningoseptica, Achromobacter species, Acidovorax* species*, Alcaligenes* species*, Brevundimonas* species*, Comamonas* species*, Methylobacterium* species*, Ochrobactrum* species, *Pandoraea* species*, Ralstonia* species*, Roseomonas* species*, Shewanella* species

# 3 Identification

## 3.1 Microscopic Appearance

**Gram stain** (TP 39 - Staining Procedures)

***Pseudomonas* species**

Gram negative rods which are straight or slightly curved. Certain strains of *Pseudomonas putida* can appear elongated.

**Note:** Organisms from older cultures may appear slightly pleomorphic.

***Stenotrophomonas maltophilia***

Gram negative straight or slightly curved non-sporulating rods.

***Burkholderia mallei***

Cells may be straight or slightly curved with rounded ends and wavy sides. The bacilli may be arranged singly, in pairs end to end, in parallel bundles or palisades.

***Burkholderia pseudomallei***

Gram negative bacilli and may appear as long bundles, but actually these represent chains of densely packed organisms. In clinical material, the staining may be irregular and bipolar staining may be seen.

***Acinetobacter* species**

Short Gram negative rods/coccobacilli, that often becomes coccoid and appearing as diplococci. They may not readily decolourise on Gram staining and demonstrate variable stain retention, along with pleomorphic variations in cell size and arrangement.

***Oligella* species**

Gram negative small rods or coccobacilli, often occurring in pairs. Cells lack the typical plumpness of *Moraxella* species.

***Psychrobacter* species**

Gram negative rods, often coccobacilli. Usually occur in planes with one plane of division. Microscopy can differentiate *Brucella* species (very small coccobacilli) from *Psychrobacter phenylpyruvicus*.

For other morphologically similar organisms, see the section on characteristics for their appearance on Gram stain.

## 3.2 Primary Isolation Media

Pseudomonas selective agar containing Cetrimide incubated in air at 35-37°C for 16–48hr.

**Note:** Agar containing Cetrimide has been used successfully to isolate and also to detect the presence of low numbers of *Pseudomonas aeruginosa* from contaminated clinical specimens[100].

Burkholderia cepacia selective agar incubated in air at 35-37°C for 48-72hr, then at 30°C for up to 5 days if necessary.

**Note:** Currently, there are no commercially available selective media specifically produced for the isolation and identification of *B. mallei* or *B. pseudomallei*[101].

Blood or chocolate agar incubated in 5-10% $CO_2$ at 35-37°C for 16-48hr.

CLED/ MacConkey agar incubated in air at 35-37°C for 16-48hr.

## 3.3 Colonial Appearance

The table is a summary of the organisms and their colonial morphology and pigment production on appropriate agar plate.

| **Organism** | **Growth characteristics on appropriate agar plate after incubation at 35-37°C for 16-48hr** |
|---|---|
| *P. aeruginosa* | Colonies are flat and spreading with serrated edges and a metallic sheen. Other morphologies exist, including smooth, mucoid and dwarf variants. Colonies are surrounded by blue-green pigment and fluoresce under short wavelength (254nm) ultraviolet light. Colonies may also appear pigmented blue or non-pigmented. They also have a grape-like odour. |
| *P. putida* and *P. fluorescens* | Both do not possess distinctive colony morphology or odour. |
| *P. monteilii, P. otitidis* and *P. mosselii* | Colonies are circular and non-pigmented when grown on nutrient agar. They are also non-haemolytic on blood agar. |
| *P. stutzeri* | Colonies are dry and wrinkled similar to the colonial morphology of *Burkholderia pseudomallei*. They can also pit or adhere to the agar and are buff to brown. |
| *P. mendocina* | Colonies are smooth, non-wrinkled and flat producing a brownish yellow pigment. |
| *P. alcaligenes* and *P. pseudoalcaligenes* | They are both non-pigmented and do not have a distinct colony morphology. |
| *P. luteola* and *P. oryzihabitans* | Colonies typically exhibit rough, wrinkled and adherent or, more rarely smooth colonies. They can both be distinguished from other pseudomonads by their negative oxidase reaction and production of non-diffusible yellow pigment. |
| *Burkholderia cepacia complex** | Colonies are circular, entire and 1-2mm in diameter with the medium turning pink. |
| *Burkholderia mallei* | On nutrient agar, colonies are smooth, grey and translucent. On MacConkey agar, growth is variable. |
| *Burkholderia pseudomallei* | On nutrient agar, rough corrugated colonies resembling *P. stutzeri* may be produced and cultures often have a pearly sheen, although there is considerable colonial variation. Some strains may produce dry and wrinkled colonies whereas others may be frankly mucoid. Usually, the colonies are not coloured, but occasional strains may produce a yellow pigment. |
| *Stenotrophomonas maltophilia* | Colonies may appear yellow or green on blood agar. Some strains may produce slight beta-haemolysis. |
| *Acinetobacter* species | Colonies are normally smooth, sometimes mucoid, pale yellow to greyish-white and some |

| | |
|---|---|
| | environmental strains may produce a diffusible brown pigment. |
| *Acidovorax* species | Colonies exhibit a creamy yellow colour, rough and raised appearance and are approximately 1.5-2.5mm in diameter. On nutrient agar, colonies are non-pigmented. |
| *Achromobacter* species | Colonies are slightly convex, translucent and non-pigmented, with smooth margins and about 0.5–2mm in diameter after 48hr incubation. |
| *Alcaligenes* species | Colonies have a thin, spreading irregular edge. |
| *Brevundimonas* species | Colonies of *B. diminuta* on MacConkey agar are chalk white, whereas colonies of *B. vesicularis* are characterised by an orange intracellular pigment. |
| *Delftia* species | On MacConkey agar, there is growth of non-pigmented, lactose non-fermenting colonies. |
| *Elizabethkingia* species | On blood agar, colonies appear as very pale yellow, pigmented and may not be evident at 24hr. |
| *Comamonas* species | On blood agar, colonies appear pink-pigmented with a mucoid and bulgy surface. They show no haemolysis on both blood and chocolate agar. |
| *Methylobacterium* species | On blood agar, colonies grow slowly. They are dry and appear pink or coral in incandescent light |
| *Ochrobactrum* species | On blood agar, colonies are 1mm in diameter after 24hr incubation and appear circular, low convex, smooth, and shining. Mucoid colonies may be produced on some media. |
| *Oligella* species | Colonies are small, white, opaque, entire and non-haemolytic after 24hr incubation. No pigments or odour are produced. |
| *Pandoraea* species | Colonies are white, circular and convex with clear margins. |
| *Psychrobacter* species | Colonies are cream-coloured, non-pigmented, small, smooth and opaque on blood agar. They require incubation at 20°C – 25°C although some strains have an optimal growth temperature of 35 - 37°C. Growth is enhanced by bile salts or Tween 80. |
| *Ralstonia* species | Colonies resembles Bcc on selective agar. They are non-pigmented. |
| *Roseomonas* species | On blood agar, colonies are pinpoint, pale pink, shiny, raised, and often mucoid after 2-3 days' incubation at 35-37°C. They produce a red-pink pigment. |
| *Shewanella* species | Colonies are distinctive smelling and produce an orange-tan pigment on blood agar. |
| *Sphingobacterium* species | Colonies produce yellow pigment. |
| **Candida* species, *S. maltophilia, R. pickettii, Pseudomonas aeruginosa, some Pseudomonas* species and | |

many other colistin resistant Gram negative bacteria (such as eg *A. johnsonii*) may also grow occasionally on Burkholderia cepacia selective agar. Consult manufacturer's guidance regarding appearance on other media.

## 3.4 Test Procedures

### 3.4.1 Oxidase test (TP 26 - Oxidase Test)

*P. aeruginosa* is oxidase positive. Other morphologically similar organisms may be oxidase positive or negative as described under the "Characteristics" section.

### 3.4.2 Commercial identification Systems

All identification tests should ideally be performed from non-selective agar. Laboratories should follow manufacturer's instructions and rapid tests and kits should be validated and be shown to be fit for purpose prior to use.

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

Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS), which can be used to analyse the protein composition of a bacterial cell, has emerged as a new technology for species identification. This has been shown to be a rapid and powerful tool because of its reproducibility, speed and sensitivity of analysis. The advantage of MALDI-TOF as compared with other identification methods is that the results of the analysis are available within a few hours rather than several days. The speed and the simplicity of sample preparation and result acquisition associated with minimal consumable costs make this method well suited for routine and high-throughput use[102].

This has been utilized to aid in both the detection and species-level identification of *Pseudomonas* species, *S. maltophilia, Acinetobacter* species, *Burkholderia mallei* and *Burkholderia pseudomallei*. It has also proven to be more discriminatory for members of the genus *Achromobacter* and *Pandoraea,* in addition to better identifying specific members of the *Burkholderia cepacia* complex of organisms[103-105].

Despite its accuracy identification, MALDI-TOF MS database needs to be updated and enlarged with a wider range of microbial species including infrequent or rare organisms recovered from patients[104].

However, MALDI-TOF MS has been unsuccessful in the identification of members of the genus *Ralstonia* and *Sphingobacterium spiritivorum* due to the absence of suitable reference spectra within the database[103].

### 3.4.4 Nucleic Acid Amplification Tests (NAATs)

PCR is usually considered to be a good method for bacterial detection as it is simple, rapid, sensitive and specific. The basis for PCR diagnostic applications in microbiology is the detection of infectious agents and the discrimination of non-pathogenic from pathogenic strains by virtue of specific genes. However, it does have limitations. Although the 16S rRNA gene is generally targeted for the design of species-specific PCR primers for identification, designing primers is difficult when the sequences of the homologous genes have high similarity.

PCR has been used for the rapid and reliable identification of *P. aeruginosa* as well as differentiation from other phylogenetically closely related *Pseudomonas* species – these include *P. resinovorans, P. alcaligenes, P. oleovorans, P. pseudoalcaligenes,*

*P. mendocina* and *P. flavescens*, all of which cluster within the *P. aeruginosa* group[106]. This method has also shown that *P. aeruginosa* can be detected at an early stage in sputum of cystic fibrosis patients and sooner than in the culture detection[107].

This has been used successfully for the rapid and reliable detection of pathogenic *Burkholderia* species – *Burkholderia mallei* and *Burkholderia pseudomallei*. The high reliability and sensitivity of the PCR assay has also made it very useful for screening of samples containing few organisms and potential inhibitors, as is the case in many environmental and clinical samples[108].

The use of PCR assays has also allowed the separation of members of the genus *Pandoraea* from closely related genera and most of all; it has allowed the accurate identification of *Pandoraea* species (all the 5 named species in the "Characteristics" section) that cause infections in humans[109]. This has also been used for the rapid and accurate identification of *Ralstonia pickettii* and *Ralstonia mannitolilytica*[65].

## 3.5 Further Identification

### Rapid Molecular Methods

Molecular methods have had an enormous impact on the taxonomy of *Pseudomonas.* Analysis of gene sequences has increased understanding of the phylogenetic relationships of *Pseudomonas* and related organisms; and has resulted in the recognition of numerous new species. Molecular techniques have made identification of many species more rapid and precise than is possible with phenotypic techniques.

Another advantage of using these rapid molecular methods is that it reduces exposure of laboratory personnel to potentially infectious samples for example; *B. pseudomallei* and *B. mallei* which are both listed as Hazard group 3 pathogens[110].

A variety of rapid typing methods have been developed for isolates from clinical samples; these include molecular techniques such as Pulsed Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), Multiple-Locus Variable-Number Tandem-Repeat Analysis (MVLA), SNP assays and Whole Genome Sequencing (WGS). All of these approaches enable subtyping of unrelated strains, but do so with different accuracy, discriminatory power, and reproducibility.

However, some of these methods remain accessible to reference laboratories only and are difficult to implement for routine bacterial identification in a clinical laboratory.

### Pulsed Field Gel Electrophoresis (PFGE)

PFGE detects genetic variation between strains using rare-cutting restriction endonucleases, followed by separation of the resulting large genomic fragments on an agarose gel. PFGE is known to be highly discriminatory and a frequently used technique for outbreak investigations and has gained broad application in characterizing epidemiologically related isolates. However, due to its time-consuming nature (30hr or longer to perform) and its requirement for special equipment, PFGE is not used widely outside the reference laboratories[111,112].

Although other typing schemes have been developed and show a variety of discriminatory powers, PFGE is known to be the gold standard for the molecular typing of *P. aeruginosa*. This technique discriminates *P. aeruginosa* isolates further than MLST would[113].

This has been used to successfully identify *Burkholderia cenocepacia*, known to infect cystic fibrosis patients[97].

### Multilocus Sequence Typing (MLST)

MLST measures the DNA sequence variations in a set of housekeeping genes directly and characterizes strains by their unique allelic profiles. The principle of MLST is simple: the technique involves PCR amplification followed by DNA sequencing. Nucleotide differences between strains can be checked at a variable number of genes depending on the degree of discrimination desired. The technique is highly discriminatory, as it detects all the nucleotide polymorphisms within a gene rather than just those non-synonymous changes that alter the electrophoretic mobility of the protein product. One of the advantages of MLST over other molecular typing methods is that sequence data are portable between laboratories and have led to the creation of global databases that allow for exchange of molecular typing data via the Internet[114].

MLST has been extensively used as the one of the main typing methods for analysing the genetic relationships within populations and this has shown that *Pseudomonas aeruginosa* has a non-clonal epidemic population structure. This technique will also aid in a better understanding of the epidemiology of *P. aeruginosa*[115].

This has been used successfully for the characterization of isolates of *B. pseudomallei* and *B. mallei.* It also found that the *B. mallei* sequence type was grouped within the *B. pseudomallei* sequence types, supporting the idea that *B. mallei* is a clone of *B. pseudomallei*. MLST appears to also have sufficient discriminatory power for epidemiological investigations of melioidosis[116].

This technique has also been used to elucidate the taxonomy and population structure of the genus *Achromobacter* as well as assign strains to defined or new genogroups[117].

MLST has equally being used for strain typing and to clearly differentiate all existing species in the closely related *B. cepacia* complex. The ability of this technique to carry out both strain differentiation and species identification in a single approach represents a major advance that should greatly aid the clinical diagnosis of *B. cepacia complex* infection[118].

This technique has also been used to study the population structure of *Stenotrophomonas maltophilia* and to unravel the uneven distribution of environmental and clinical isolates obtained from infected, colonized, or CF patients. The MLST data developed has also confirmed the existence of previously defined genogroups and also identified new genogroups as well. However, further taxonomic studies are required to assess whether *S. maltophilia* can be separated into several distinct species[119].

The drawbacks of MLST are the substantial cost and laboratory work required to amplify, determine, and proof read the nucleotide sequence of the target DNA fragments, making the method hardly suitable for routine laboratory testing.

### 16S rRNA Gene Sequencing

A genotypic identification method, 16S rRNA gene sequencing is used for phylogenetic studies and has subsequently been found to be capable of re-classifying bacteria into completely new species, or even genera. It has also been used to describe new species that have never been successfully cultured.

Although 16S rRNA gene sequencing is highly useful in regards to bacterial classification, it has low phylogenetic power at the species level and poor

discriminatory power for some genera, and DNA relatedness studies are necessary to provide absolute resolution to these taxonomic problems. There have been reports of resolution problems at the genus and/or species level with this technique[120]. Examples of these groups include (not exclusively), the *Acinetobacter baumannii-A. calcoaceticus* complex, *Achromobacter*, *Stenotrophomonas*, and some *Pseudomonas* species (*P. fluorescens*, *P. jessenii*)[121].

This has been used to accurately identify and differentiate between *Burkholderia mallei* and *Burkholderia pseudomallei* [110]. This has equally been used to describe the *Pandoraea* species as well as *Roseomonas* species[60,70].

### *gyrB* Gene Sequencing

The *gyrB* gene, encoding the subunit B of DNA gyrase (a type II topoisomerase), has been demonstrated to represent a useful molecular marker for phylogenetic and/or taxonomic analysis. It has been used successfully in the identification of genera such as *Pseudomonas*, *Acinetobacter*, *Pandorea* and *Stenotrophomonas*. It has also been found to be a useful tool to discriminate strains belonging to the Bcc complex[122,123].

### *recA* Gene Sequencing

In contrast to the 16S rRNA gene, which has only limited taxonomic resolution in the Bcc, the *recA* gene has mostly provided the discriminatory power needed for identification of Bcc species and has been used successfully for the identification and differentiation of *B. pseudomultivorans* from other Bcc species[14].

### Whole Genome Sequencing (WGS)

This is also known as “full genome sequencing, complete genome sequencing, or entire genome sequencing”. It is a laboratory process that determines the complete DNA sequence of an organism's genome at a single time. There are several high-throughput techniques that are available and used to sequence an entire genome such as pyrosequencing, nanopore technology, IIlumina sequencing, Ion Torrent sequencing, etc. This sequencing method holds great promise for rapid, accurate, and comprehensive identification of bacterial transmission pathways in hospital and community settings, with concomitant reductions in infections, morbidity, and costs.

This has been used successfully to explore the genome of *Pseudomonas* species. In the year 2000, the complete genome sequence of *Pseudomonas aeruginosa* was determined[124]. This was of interest because of the insights it provided into the role of this bacterium as a pathogen. More recently, the sequences of other *Pseudomonas* strains have been determined.

This has also been used for deducing the phylogeny of more closely related *Burkholderia* species, such as members of the *B. cepacia* complex or the *B. mallei* lineage[13].

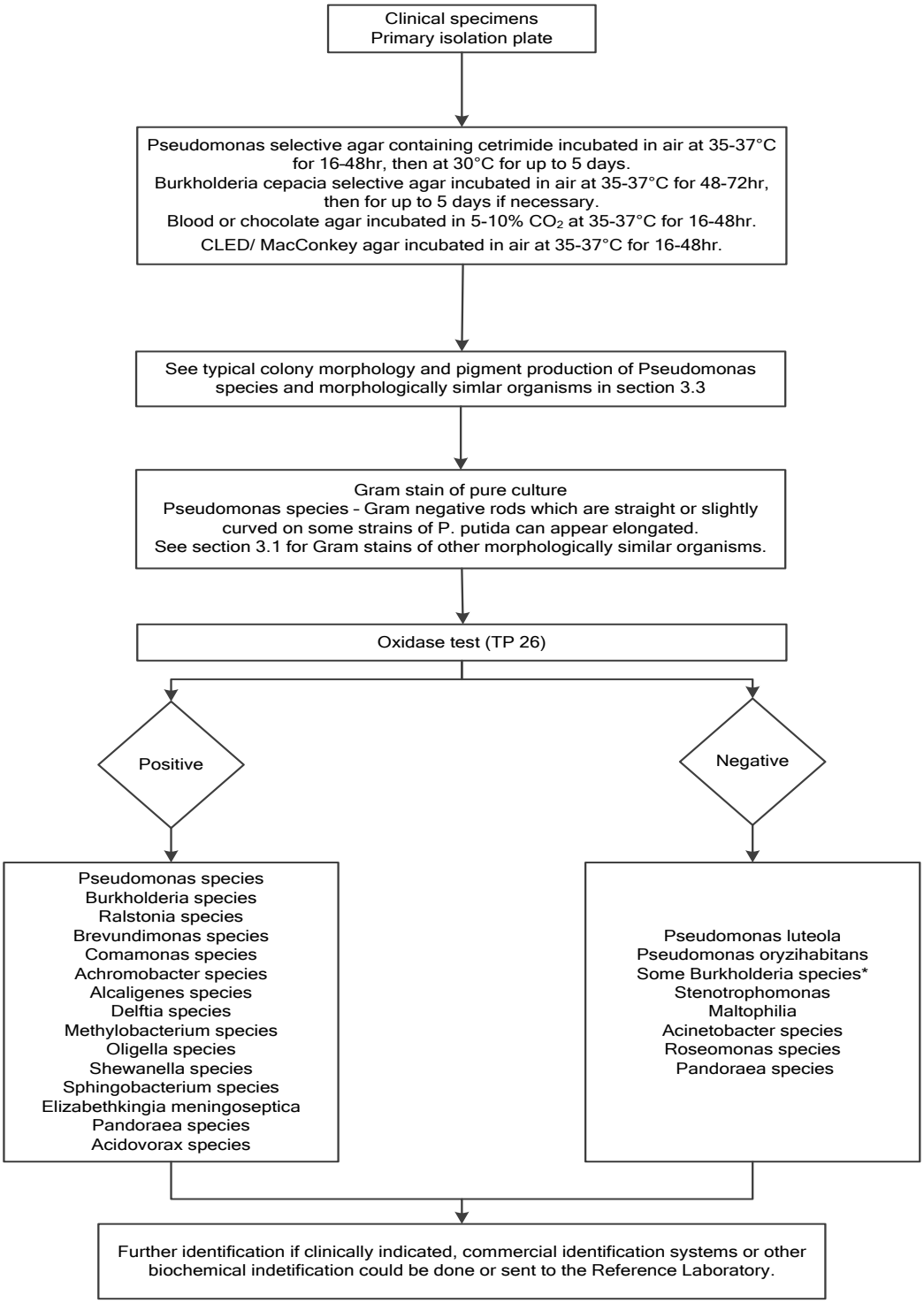
WGS has also been used successfully to explore the genome of *Stenotrophomonas maltophilia,* whose sequence reveals that the organism can act as a reservoir of antimicrobial drug resistance determinants in clinical environments, which is an issue of considerable concern. This also reveals the capacity of this organism for environmental adaptations that presumably contribute to its persistence *in vivo* for example is its strong ability to attach to catheters and ventilators, from which infections of the blood or lungs arise[125].

This has also been used to explore the draft genome sequences of *Acinetobacter* species - *A. baumannii, A. nosocomialis*, and *A. pittii* associated with nosocomial infections[126].

## 3.6 Storage and Referral

If required, save isolate on blood or nutrient agar slopes or charcoal swabs for referral to the Reference Laboratory.

# 4 Identification of *Pseudomonas* species and other Non-Glucose Fermenters




*Some Burkholderia species give variable reactions on oxidase test (B. contaminans, B. lata, B. pyrrocinia, B. gladioli, B. mallei)

The flowchart is for guidance only.

# 5 Reporting

## 5.1 Presumptive Identification

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

## 5.2 Confirmation of Identification

Further biochemical tests and/or molecular methods and/or Reference Laboratory report.

## 5.3 Medical Microbiologist

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 *Burkholderia pseudomallei* eg septicaemia, pneumonia, or multi-system disease with abscess formation (and possible outbreaks of same) in association with:

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

*Burkholderia mallei* may present with somewhat similar clinical features, in association with:

- Agricultural/livestock, veterinary or laboratory work overseas, especially in the Middle East and S. America.

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. If isolated from other site(s), consideration should be given to informing the medical microbiologist in accordance with local protocols, if eg:

- Immuno-compromised patient notably if neutropenic
- Device-associated infection

The medical microbiologist should be informed of presumed or confirmed *Burkholderia cepacia* complex isolates from cystic fibrosis patients.

Follow local protocols for reporting to clinician.

## 5.4 CCDC

Refer to local Memorandum of Understanding.

## 5.5 Public Health England[127]

Refer to current guidelines on CIDSC and COSURV reporting.

### 5.6 Infection Prevention and Control Team

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

# 6 Referrals

### 6.1 Reference Laboratory

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

Antimicrobial Resistance and Healthcare Associated Infections Reference Unit (AMRHAI)
Public Health England
61 Colindale Avenue
London
NW9 5EQ
Contact PHE's main switchboard: Tel. +44 (0) 20 8200 4400

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.belfasttrust.hscni.net/Laboratory-MortuaryServices.htm

# 7 Notification to PHE[127,128] or Equivalent in the Devolved Administrations[129-132]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

**Note:** The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[129,130], Wales[131] and Northern Ireland[132].

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