



# UK Standards for Microbiology Investigations

## Identification of Glucose Non-Fermenting Gram Negative Rods



## 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 <http://www.hpa.org.uk/SMI/Partnerships>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <http://www.hpa.org.uk/SMI/WorkingGroups>).

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

For full details on our accreditation visit: [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation).

## 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](mailto: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.	4/11.03.14
Issue no. discarded.	2.1
Insert Issue no.	2.2
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	<p>Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England.</p> <p>Front page has been redesigned.</p> <p>Status page has been renamed as Scope and Purpose and updated as appropriate.</p> <p>Professional body logos have been reviewed and updated.</p> <p>Standard safety and notification references have been reviewed and updated.</p> <p>Scientific content remains unchanged.</p>

Amendment No/Date.	3/21.10.11
Issue no. discarded.	2
Insert Issue no.	2.1
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	Document presented in a new format.
References.	References updated.

# UK Standards for Microbiology Investigations<sup>#</sup>: Scope and Purpose

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## 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

<http://www.hpa.org.uk/SMI/Partnerships>. 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.

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<sup>#</sup>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

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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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**Suggested Citation for this Document**

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UNDER REVIEW

## Scope of Document

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This SMI includes the identification of *Pseudomonas* species, *Burkholderia* species and other glucose non-fermenting Gram negative bacilli that have been associated with human infection<sup>1</sup>. It describes the identification of *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex (Bcc) 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<sup>2</sup>.

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”. 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.

Non-fermenting Gram negative bacilli 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.

This SMI should be used in conjunction with other SMIs.

## Introduction

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### Taxonomy<sup>1,3,4</sup>

The taxonomic status of many strains in this large heterogeneous group of organisms is undergoing continuous revision. Organisms previously classified within the genus *Pseudomonas* (rRNA homology groups I-V) are now divided among the genera *Pseudomonas*, *Burkholderia*, *Ralstonia*, *Comamonas*, *Brevundimonas* and *Stenotrophomonas*. 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 aeruginosa*<sup>1</sup>

*P. aeruginosa* is the glucose non-fermenting Gram negative rod most often associated with human infection. It has the characteristic 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 pyoverdinin (fluorescein, yellow). Production of blue-green pigment is indicative of *P. aeruginosa*<sup>5</sup>. Some strains produce other pigments, such as pyorubin (red) or pyomelanin (brown).

*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 sensitivity.

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 37°C or room temperature for 48hr so that their phenotypic features may reliably be expressed.

### **Other *Pseudomonas* species**

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<sup>6</sup>. 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 two species by its inability to liquefy gelatine<sup>6</sup>.

*Pseudomonas caryophylli* produces smooth, intermediate and rough colonies (sometimes yellow pigmented) when grown on nutrient agar<sup>7</sup>. The latter can resemble colonies of *Burkholderia pseudomallei* or *Bacillus* species.

*Pseudomonas alcaligenes* and *Pseudomonas pseudoalcaligenes* are both non-pigmented.

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.

### ***Burkholderia cepacia* complex**

Recent research has resulted in a number of changes to the taxonomy of *Burkholderia cepacia* complex (Bcc). Briefly, nucleotide analysis of *recA* gene sequences suggests

that Bcc consists of nine closely related genomovars<sup>8</sup>. Most of these have now been classified as individual species (*B. cepacia*, *B. multivorans*, *B. stabilis*, *B. vietnamiensis*, *B. ambifaria*, *B. athina*, *B. pyrrocinia*). This is important as 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 Cystic Fibrosis (CF) units)<sup>9,10</sup>. Some Bcc strains may be isolated from contaminated medical devices such as blood gas analysers, nebuliser equipment or disinfectants<sup>11-13</sup>.

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<sup>14,15</sup>. All contain antibiotics to improve selectivity. Media should be incubated at 35–37°C for two days. Some strains may appear only if the plates are further incubated at 20°C for up to five days. Colonial appearances vary according to the medium employed. It is important that presumed 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<sup>16,17</sup>. 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 melioidosis.

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. Isolates of *B. pseudomallei* are constitutively resistant to polymyxin and aminoglycosides, but susceptible to co-amoxiclav. *P. stutzeri* and *B. pseudomallei* may have similar colonial appearances See the [HPA website](#) for pictures of the colonies. Suspect colonies should be referred to the Reference Laboratory. 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* grows readily on media containing polymyxin. Unlike Bcc, they are oxidase negative and do not oxidise maltose and lactose. *B. gladioli* is occasionally isolated from the respiratory tract of patients with CF but, unlike Bcc, its clinical significance in these patients remains uncertain. Molecular methods may be required to confirm its identity<sup>18</sup>.

### ***Stenotrophomonas maltophilia***<sup>19</sup>

Numerically *S. maltophilia* is the second most commonly isolated glucose non-fermenter in clinical laboratories after *P. aeruginosa*. 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 slowly oxidase positive. 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.

### ***Acinetobacter* species**<sup>20</sup>

Based on DNA-DNA hybridisation studies, there are now at least 19 different *Acinetobacter* genomic species. Seven of these have been given species names, namely *A. calcoaceticus*, *A. baumannii*, *A. haemolyticus*, *A. junii*, *A. johnsonii*, *A. Iwoffii*, 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. Iwoffii*, *A. johnsonii* and *A. haemolyticus*.

*Acinetobacter* species are short Gram negative rods/coccobacilli, typically 1.0-1.5 x 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<sup>21</sup>. Colonies are normally smooth, sometimes mucoid, pale yellow to greyish-white and some environmental strains may produce a diffusible brown pigment. Colony size is similar to that of the Enterobacteriaceae, from which they need to be distinguished. *A. Iwoffii* 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.

*Acinetobacter* species are strict aerobes, oxidase negative, catalase positive, non-motile and non-fermentative. Some clinical isolates, particularly *Acinetobacter haemolyticus*, may be haemolytic on blood agar. 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.

## Other non-fermenters

There are many other glucose non-fermenting Gram negative bacilli 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 immunocompromised. 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<sup>22,23</sup>. In such cases it may be appropriate to refer these isolates to a Reference Laboratory.

Non-fermenting Gram negative bacilli isolated occasionally from clinical specimens include:

### ***Achromobacter (Alcaligenes) xylosoxidans***

*Alcaligenes xylosoxidans* was reclassified as *Achromobacter xylosoxidans* in 1998<sup>24</sup>. It has occasionally been isolated from respiratory secretions of patients with CF and has also caused sepsis in other patients who are immuno-compromised<sup>25,26</sup>. It is both catalase and oxidase positive.

### ***Alcaligenes* species**

*Alcaligenes faecalis* is the type species<sup>27</sup>. Colonies have a thin, spreading irregular edge. It is catalase negative, oxidase positive and motile.

### ***Bordetella* species**

For information on *Bordetella* species please see [ID 5 - Identification of \*Bordetella pertussis\* and \*Bordetella parapertussis\* from Selective Agar](#).

### ***Brevundimonas* species**

*Brevundimonas vesicularis* and *Brevundimonas diminuta* grow slowly on ordinary nutrient media<sup>28</sup>. Unlike *B. diminuta*, *B. vesicularis* gives only a weak oxidase reaction. It forms a carotenoid pigment that produces yellow or orange colonies.

### ***Elizabethkingia* species**

*Elizabethkingia* (formerly *Chryseobacterium*) *meningosepticum*, 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%<sup>29,30</sup>. The organism produces very pale yellow pigmented colonies on blood agar that may not be evident at 24hr (in contrast to the more commonly isolated dark yellow colonies of *E. indologenes*). *E. meningosepticum* is non-motile and oxidase positive. It 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. *E. indologenes* is also non-motile and oxidase positive<sup>31</sup>.

### ***Comamonas* species**

*Comamonas terrigena* is the type species. It is motile, oxidase and catalase positive. *Comamonas acidovorans* characteristically produces an orange indole reaction due to anthranilic acid rather than indole production from tryptophan.

### **Methylobacterium species**

*Methylobacterium* species colonies grow slowly on blood agar, are dry and appear pink or coral in incandescent light<sup>32</sup>. 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.

### **Moraxella species**

For information on *Moraxella* species please see [ID 11 - Identification of Moraxella species and Morphologically Similar Organisms](#).

### **Ochrobactrum species**

*Ochrobactrum anthropi* is urease positive, aesculin and ONPG negative. Colonies are 1mm in diameter on blood agar after 24hr incubation, and appear circular, low convex, smooth, and shining<sup>33</sup>. Muroid colonies may be produced on some media.

### **Oligella species**

*Oligella ureolytica* grows slowly on blood agar producing pinpoint colonies after 24hr, and large colonies only after three days incubation. Colonies are white, opaque, entire and non-haemolytic. It is oxidase positive and motile. *Oligella urethralis* is similar to *Moraxella* and *Acinetobacter* species in that isolates are coccobacillary, oxidase negative and non-motile.

### **Psychrobacter species**

*Psychrobacter immobilis* and *Psychrobacter phenylpyruvicus* (previously *Moraxella*) cells are coccoid to short, thick rods which may be vacuolated and stain peripherally. It is oxidase positive and non-motile. Growth is optimal at 20°C and poor at 37°C.

### **Ralstonia species**

*Ralstonia pickettii* (formerly *Burkholderia pickettii*) is non-pigmented, oxidase positive, and will grow at 4°C, but does not attack arginine. It resembles Bcc on selective agar and can be difficult to distinguish from it biochemically<sup>34</sup>.

### **Roseomonas species**

*Roseomonas* species produce red-pink pigment and cells appear as coccoid rods in pairs or short chains or may be mainly cocci with occasional rods<sup>35</sup>. Growth on blood agar is pinpoint, pale pink, shiny, raised, and often mucoid after two to three days incubation at 35-37°C. They are weakly oxidase positive or oxidase negative, catalase positive and urease positive. The genus comprises six species of which four are reported to cause infection.

### **Shewanella species**

*Shewanella putrefaciens* is oxidase positive and motile. Colonies are distinctive smelling and produce an orange-tan pigment on blood agar<sup>36</sup>.

### **Sphingobacterium species**

They are oxidase positive and non-motile. Colonies produce yellow pigment. The species isolated most frequently from clinical specimens are *Sphingobacterium multivorum* and *Sphingobacterium spiritivorum*.

## Principles of Identification

Isolates from primary culture are identified by colonial appearance, Gram stain, and preliminary tests, which permit the presumptive identification of *P. aeruginosa*. Additional identification may be made using a commercial identification kit.

## Technical Information/Limitations

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Basic commercial identification 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. Few systems identify Bcc accurately, and other organisms such as *S. maltophilia* may be misidentified as Bcc. 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.

UNDER REVIEW

## 1 Safety Considerations<sup>37-53</sup>

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*B. mallei* and *B. pseudomallei* are Hazard Group 3 organisms, and 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.

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.

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

Compliance with postal and transport regulations is essential.

## 2 Target Organisms

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**Glucose non-fermenting Gram negative bacilli, commonly isolated in the clinical laboratory**

*Acinetobacter species*

*Burkholderia cepacia complex*

*Pseudomonas aeruginosa*

*Stenotrophomonas maltophilia*

**Hazard Group 3 pathogens**

*Burkholderia mallei*

*Burkholderia pseudomallei*

## 3 Identification

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### 3.1 Microscopic Appearance

**Gram stain ([Table 39 - Staining Procedures](#))**

Gram negative rods

### 3.2 Primary Isolation Media

*Pseudomonas* selective agar, 16–48hr incubation in air at 35°C-37°C, then at 30°C for up to five days.

*Burkholderia cepacia* selective agar, 16–48hr incubation in air at 35°C-37°C, then at 30°C for up to five days.

Blood or chocolate agar, 16-48hr incubation in CO<sub>2</sub>, at 35°C-37°C.

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

### 3.3 Colonial Appearance

Colonies of *B. cepacia* complex on *Burkholderia cepacia* selective agar are 1-2mm in diameter with the medium turning pink. *Candida* species, *S. maltophilia*, *R. pickettii*, some *Pseudomonas* species and many other colistin-resistant Gram negative bacteria may also grow on this medium, eg *A. johnsonii*. Consult manufacturer's guidance regarding appearance on other media.

Colonies of *P. aeruginosa* on *Pseudomonas* selective agar are surrounded by blue-green pigment, and fluoresce under short wavelength (254nm) ultraviolet light.

Colonial morphology

Pigment production

### 3.4 Test Procedures

#### Oxidase test ([TP 26 - Oxidase Test](#))

*P. aeruginosa* is oxidase positive. Other glucose non-fermenting rods may be oxidase positive or negative as described.

#### Commercial identification kit

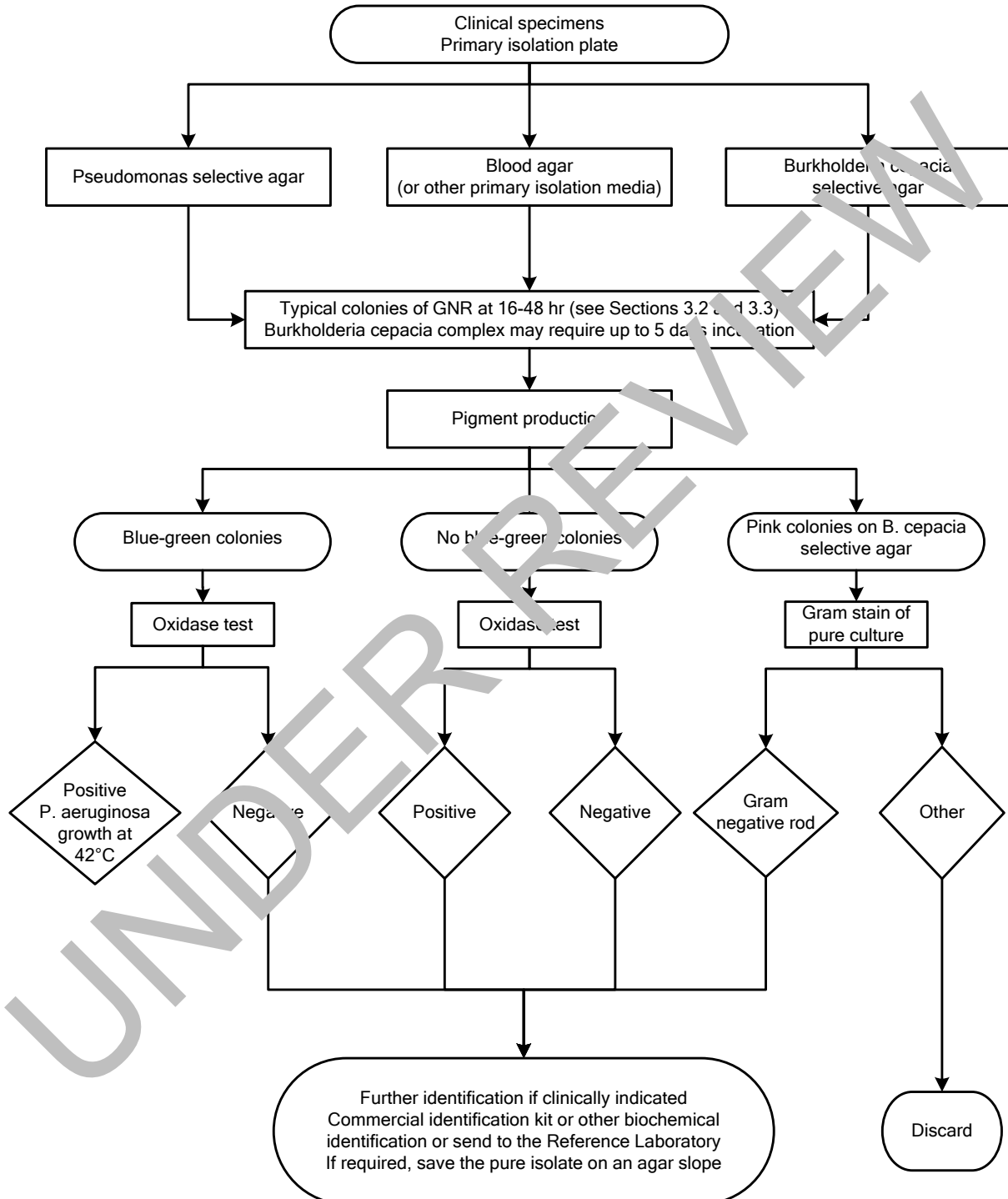
### 3.5 Further Identification

Following commercial identification test kit results and/or the Reference Laboratory report.

### 3.6 Storage and Referral

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

## 4 Identification of Glucose Non-Fermenting Gram Negative Rods



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 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 eg:

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

Presumed or confirmed *Burkholderia cepacia* complex isolates from cystic fibrosis patients.

The medical microbiologist should be informed if the request card 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
- laboratorian 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

Follow local protocols for reporting to clinician.

### 5.4 CCDC

Refer to local Memorandum of Understanding.

### 5.5 Public Health England<sup>54</sup>

Refer to current guidelines on CDSC and COSURV reporting.

## 5.6 Infection Control Team

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

## 6 Referrals

### 6.1 Reference Laboratory

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

Antimicrobial Resistance and Healthcare Associated Infections Reference Unit (AMRHAI)

Microbiology Services Division

Public Health England

61 Colindale Avenue

London

NW9 5EQ

Contact PHE's main switchboard: Tel. +44 (0) 20 8200 4000

England and Wales

<http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1158313434370?p=1158313434370>

Scotland

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

Northern Ireland

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

## 7 Notification to PHE<sup>54,55</sup> or Equivalent in the Devolved Administrations<sup>56-59</sup>

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 1 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 (HCAs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

Other arrangements exist in Scotland<sup>56,57</sup>, Wales<sup>58</sup> and Northern Ireland<sup>59</sup>.

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