

NATIONAL STANDARD METHOD

# IDENTIFICATION OF GLUCOSE NON-FERMENTING GRAM- NEGATIVE RODS

BSOP ID 17

Issued by Standards Unit, Evaluations and Standards Laboratory  
Centre for Infections

*Association of Medical Microbiologists*  
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**IDENTIFICATION OF GLUCOSE NON-FERMENTING GRAM-NEGATIVE RODS**

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# AMENDMENT PROCEDURE

<b>Controlled document reference</b>	<b>BSOP ID 17</b>
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Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from [standards@hpa.org.uk](mailto:standards@hpa.org.uk).

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
2/ 02.04.07	1.1	2	All	<b>All</b>	Taxonomy updated
			13	<b>Flow chart</b>	Title changed and flowchart put in to visio format. Contents of flow chart updated.
			14	<b>5.3 Medical Microbiologist</b>	Section amended to make it more informative in the work place.
			15	<b>6 Referrals</b>	Links to reference laboratory user manuals inserted.
			16	<b>References</b>	References reviewed and updated

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# IDENTIFICATION OF GLUCOSE NON-FERMENTING GRAM-NEGATIVE RODS

## SCOPE OF DOCUMENT

This National Standard Method (NSM) includes the identification of *Pseudomonas* species, *Burkholderia* species and other glucose non-fermenting Gram-negative bacilli that have been associated with human infection<sup>2</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 NSM 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>3</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.

## INTRODUCTION

### TAXONOMY<sup>2,4,5</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>2</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°C - 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 pyoverdine (fluorescein, yellow). Production of blue-green pigment is indicative of *P. aeruginosa*<sup>6</sup>. Some strains produce other pigments, such as pyorubin (red) or pyomelanin (brown).

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*P. aeruginosa* can produce at least six colonial types after aerobic incubation on nutrient agar for 24 h 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 48 hours 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>7</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>7</sup>.

*Pseudomonas stutzeri* produces smooth, intermediate and rough colonies (sometimes yellow pigmented) when grown on nutrient agar<sup>8</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>9</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<sup>10</sup> (eg *B. cepacia* genomovar III and outbreaks of fulminant pneumonitis in CF units<sup>11</sup>). Some Bcc strains may be isolated from contaminated medical devices such as blood gas analysers<sup>12</sup>, nebuliser equipment<sup>13</sup> or disinfectants<sup>14</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>15,16</sup>. All contain antibiotics to improve selectivity. Media should be incubated at 35°C – 37°C for two days. Some strains may appear only if the plates are further incubated at 30°C for up to five 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

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commercial identification system, although these remain unreliable for confirmation of Bcc<sup>17,18</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 [http://www.hpa.org.uk/infections/topics\\_az/deliberate\\_release/Glanders/Homepage.asp](http://www.hpa.org.uk/infections/topics_az/deliberate_release/Glanders/Homepage.asp) 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>19</sup>.

### ***Stenotrophomonas maltophilia*<sup>20</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>21</sup>**

Based on DNA-DNA hybridization 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. lwoffii*, 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. lwoffii*, *A. johnsonii* and *A. haemolyticus*.

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*Acinetobacter* species are short, Gram-negative rods/coccobacilli, typically 1.0 - 1.5 by 1.5 - 2.5 µm, 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>22</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. luoffii* and some other species are 0.5µm or less at 24-48 hours. Most strains have an optimum growth temperature of 30°C - 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 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<sup>23,24</sup> may be needed to confirm the organism's identity. 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>25</sup>. It has occasionally been isolated from respiratory secretions of patients with CF<sup>26</sup> and has also caused sepsis in other patients who are immuno-concompromised<sup>27</sup>. It is both catalase- and oxidase-positive.

#### ***Alcaligenes* species**

*Alcaligenes faecalis* is the type species<sup>28</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 BSOP 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>29</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>30,31</sup>. The organism produces very pale yellow pigmented colonies on blood agar that may not be evident at 24 hours (in contrast to the more commonly isolated dark yellow colonies of *E. indologenes*). *E. meningosepticum* is nonmotile and oxidase-positive. It hydrolyses aesculin and gelatin, is positive for the o-nitrophenyl-b-galactopyranoside (ONPG) test, and produces indole. However, the indole

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reaction is described as only weakly positive after 48 h 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 nonmotile and oxidase-positive<sup>32</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 and are dry and appear pink or coral in incandescent light<sup>33</sup>. Optimum growth occurs at 25°C - 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 BSOP ID 11 - Identification of *Moraxella* species and morphologically similar organisms.

### **Ochrobactrum species**

*Ochrobactrum anthropi* is urease-positive and aesculin- and ONPG-negative. Colonies are 1 mm in diameter on blood agar after 24 hours incubation and appear circular, low convex, smooth, and shining<sup>34</sup>. Mucoid colonies may be produced on some media.

### **Oligella species**

*Oligella ureolytica* grows slowly on blood agar producing pinpoint colonies after 24 hours 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 41°C, but does not attack arginine. It resembles Bcc on selective agar and can be difficult to distinguish from it biochemically<sup>35</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>36</sup>. Growth on blood agar is pinpoint, pale pink, shiny, raised, and often mucoid after 2 - 3 days' incubation at 35°C - 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.

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### ***Shewanella* species**

*Shewanella putrefaciens* is oxidase-positive and motile. Colonies are distinctive smelling<sup>37</sup> and produce an orange-tan pigment on blood agar.

### ***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**

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. 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.

# 1 SAFETY CONSIDERATIONS<sup>38-48</sup>

*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 NSM.

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

**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

### 3.1 MICROSCOPIC APPEARANCE

**Gram stain** (see BSOP TP 39 - Staining procedures)

Gram-negative rods

### 3.2 PRIMARY ISOLATION MEDIA

*Pseudomonas* selective agar 16 – 48 h incubation in air at 35°C to 37°C, then at 30°C for up to 5 days.

*Burkholderia cepacia* selective agar 16 – 48 h incubation in air at 35°C to 37°C, then at 30°C for up to 5 days

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

CLED agar 16 - 48 h incubation in air at 35°C to 37°C

### 3.3 COLONIAL APPEARANCE

Colonies of *B. cepacia* complex on *Burkholderia cepacia* selective agar are 1-2 mm 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 such as 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 (254 nm) ultraviolet light.

Colonial morphology

Pigment production

### **3.4 TEST PROCEDURES**

**Oxidase test** (see BSOP TP 26 - Oxidase test)

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

Further identification

Commercial identification kit

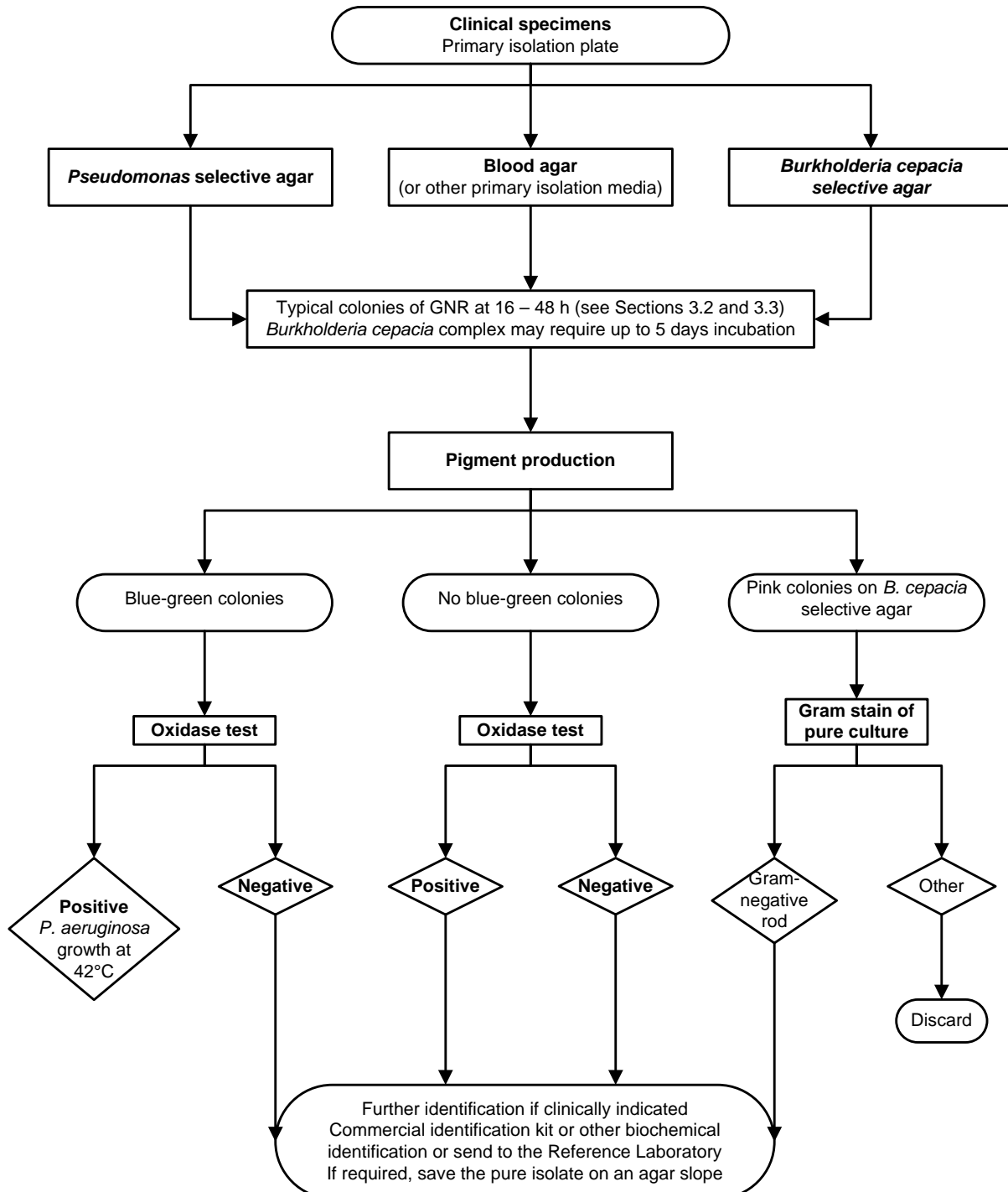
### **3.5 FUTURE IDENTIFICATION**

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

### **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 GLUCOSE NON-FERMENTING GRAM-NEGATIVE RODS - FLOWCHART



## IDENTIFICATION OF GLUCOSE NON-FERMENTING GRAM-NEGATIVE RODS

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## 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 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, if 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
- 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

Follow local protocols for reporting to clinician

### 5.4 CCDC

Refer to local Memorandum of Understanding

### 5.5 CFI<sup>49</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*.

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## 6 REFERRALS

### 6.1 REFERENCE LABORATORY

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory refer to: <http://www.hpa.org.uk/cfi/dhcaiar>

Laboratory of Health Care Association Infection  
Centre for Infections  
61 Colindale Avenue  
London  
NW9 5HT

Contact CFI main switchboard: Tel. +44 (0) 20 8200 4400

## 7 ACKNOWLEDGMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the Standard Methods Working Group for Bacteriology ([http://www.hpa-standardmethods.org.uk/wg\\_bacteriology.asp](http://www.hpa-standardmethods.org.uk/wg_bacteriology.asp)). The contributions of many individuals in clinical bacteriology laboratories and specialist organisations who have provided information and comment during the development of this document, and final editing by the Medical Editor are acknowledged.

The National Standard Methods are issued by Standards Unit, Evaluations and Standards Laboratory, Centre for Infections, Health Protection Agency London.

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# REFERENCES

1. Department of Health NHS Executive: The Caldicott Committee. Report on the review of patient-identifiable information. London. December 1997.
2. Pitt TL, Simpson AJ. *Pseudomonas aeruginosa* and *Burkholderia* spp. In: Hawkey PM, Gillespie SH, editors. Principles and Practice of Clinical Bacteriology. Chichester: John Wiley & Sons; 2006. p. 426-43.
3. Ogunnariwo J, Hamilton-Miller JM. Brown- and red-pigmented *Pseudomonas aeruginosa*: differentiation between melanin and pyorubrin. *J Med Microbiol* 1975;8:199-203.
4. Govan JRW. *Pseudomonas*, *Stenotrophomonas*, *Burkholderia*. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie & McCartney Practical Medical Microbiology. 14<sup>th</sup> ed. Edinburgh: Churchill Livingstone; 1996. p. 413-24.
5. Gilligan PH. *Pseudomonas* and *Burkholderia*. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover FC, editors. Manual of Clinical Microbiology. 6<sup>th</sup> ed. Washington D.C.: American Society for Microbiology; 1995. p. 509-19.
6. Phillips I. Identification of *Pseudomonas aeruginosa* in the clinical laboratory. *J Med Microbiol* 1969;2:9-16.
7. Hsueh PR, Teng LJ, Pan HJ, Chen YC, Sun CC, Ho SW, et al. Outbreak of *Pseudomonas fluorescens* bacteremia among oncology patients. *J Clin Microbiol* 1998;36:2914-7.
8. Noble RC, Overman SB. *Pseudomonas stutzeri* infection. A review of hospital isolates and a review of the literature. *Diagn Microbiol Infect Dis* 1994;19:51-6.
9. Mahenthalingam E, Baldwin A, Vandamme P. *Burkholderia cepacia* complex infection in patients with cystic fibrosis. *J Med Microbiol* 2002;51:533-8.
10. Govan JR, Hughes JE, Vandamme P. *Burkholderia cepacia*: medical, taxonomic and ecological issues. *J Med Microbiol* 1996;45:395-407.
11. Coenye T, Vandamme P, Govan JR, LiPuma JJ. Taxonomy and identification of the *Burkholderia cepacia* complex. *J Clin Microbiol* 2001;39:3427-36.
12. Gravel-Tropper D, Sample ML, Oxley C, Toye B, Woods DE, Garber GE. Three-year outbreak of pseudobacteremia with *Burkholderia cepacia* traced to a contaminated blood gas analyzer. *Infect Control Hosp Epidemiol* 1996;17:737-40.
13. Hutchinson GR, Parker S, Pryor JA, Duncan-Skingle F, Hoffman PN, Hodson ME, et al. Home-use nebulizers: a potential primary source of *Burkholderia cepacia* and other colistin-resistant, gram-negative bacteria in patients with cystic fibrosis. *J Clin Microbiol* 1996;34:584-7.
14. Panlilio AL, Beck-Sague CM, Siegel JD, Anderson RL, Yetts SY, Clark NC, et al. Infections and pseudoinfections due to povidone-iodine solution contaminated with *Pseudomonas cepacia*. *Clin Infect Dis* 1992;14:1078-83.
15. Henry D, Campbell M, McGimpsey C, Clarke A, Loudon L, Burns JL, et al. Comparison of isolation media for recovery of *Burkholderia cepacia* complex from respiratory secretions of patients with cystic fibrosis. *J Clin Microbiol* 1999;37:1004-7.
16. Wright RM, Moore JE, Shaw A, Dunbar K, Dodd M, Webb K, et al. Improved cultural detection of *Burkholderia cepacia* from sputum in patients with cystic fibrosis. *J Clin Pathol* 2001;54:803-5.

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17. Shelly DB, Spilker T, Gracely EJ, Coenye T, Vandamme P, LiPuma JJ. Utility of commercial systems for identification of *Burkholderia cepacia* complex from cystic fibrosis sputum culture. *J Clin Microbiol* 2000;38:3112-5.
18. Brisse S, Stefani S, Verhoef J, van Belkum A, Vandamme P, Goessens W. Comparative evaluation of the BD Phoenix and VITEK 2 automated instruments for identification of isolates of the *Burkholderia cepacia* complex. *J Clin Microbiol* 2002;40:1743-8.
19. Bauernfeind A, Schneider I, Jungwirth R, Roller C. Discrimination of *Burkholderia gladioli* from other *Burkholderia* species detectable in cystic fibrosis patients by PCR. *J Clin Microbiol* 1998;36:2748-51.
20. Denton M, Kerr KG. Microbiological and clinical aspects of infection associated with *Stenotrophomonas maltophilia*. *Clin Microbiol Rev* 1998;11:57-80.
21. Bergogne-Berezin E, Towner KJ. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. *Clin Microbiol Rev* 1996;9:148-65.
22. Pitt TL, Kaufmann ME, Patel PS, Benghe LC, Gaskin S, Livermore DM. Type characterisation and antibiotic susceptibility of *Burkholderia* (*Pseudomonas*) *cepacia* isolates from patients with cystic fibrosis in the United Kingdom and the Republic of Ireland. *J Med Microbiol* 1996;44:203-10.
23. Coenye T, Goris J, Spilker T, Vandamme P, LiPuma JJ. Characterization of unusual bacteria isolated from respiratory secretions of cystic fibrosis patients and description of *Inquilinus limosus* gen. nov., sp. nov. *J Clin Microbiol* 2002;40:2062-9.
24. Ferroni A, Sermet-Gaudelus I, Abachin E, Quesne G, Lenoir G, Berche P, et al. Use of 16S rRNA gene sequencing for identification of nonfermenting gram-negative bacilli recovered from patients attending a single cystic fibrosis center. *J Clin Microbiol* 2002;40:3793-7.
25. Clermont D, Harmant C, Bizet C. Identification of strains of *Alcaligenes* and *Agrobacterium* by a polyphasic approach. *J Clin Microbiol* 2001;39:3104-9.
26. Saiman L, Chen Y, Tabibi S, San Gabriel P, Zhou J, Liu Z, et al. Identification and antimicrobial susceptibility of *Alcaligenes xylosoxidans* isolated from patients with cystic fibrosis. *J Clin Microbiol* 2001;39:3942-5.
27. Manfredi R, Nanetti A, Ferri M, Chiodo F. Bacteremia and respiratory involvement by *Alcaligenes xylosoxidans* in patients infected with the human immunodeficiency virus. *Eur J Clin Microbiol Infect Dis* 1997;16:933-8.
28. Bizet J, Bizet C. Strains of *Alcaligenes faecalis* from clinical material. *J Infect* 1997;35:167-9.
29. Gilad J, Borer A, Peled N, Riesenberk K, Tager S, Appelbaum A, et al. Hospital-acquired *Brevundimonas vesicularis* septicaemia following open-heart surgery: case report and literature review. *Scand J Infect Dis* 2000;32:90-1.
30. Chiu CH, Waddington M, Greenberg D, Schreckenberger PC, Carnahan AM. Atypical *Chryseobacterium meningosepticum* and meningitis and sepsis in newborns and the immunocompromised, Taiwan. *Emerg Infect Dis* 2000;6:481-6.
31. Hoque SN, Graham J, Kaufmann ME, Tabaqchali S. *Chryseobacterium* (*Flavobacterium*) *meningosepticum* outbreak associated with colonization of water taps in a neonatal intensive care unit. *J Hosp Infect* 2001;47:188-92.
32. Hsueh PR, Teng LJ, Yang PC, Ho SW, Hsieh WC, Luh KT. Increasing incidence of nosocomial *Chryseobacterium indologenes* infections in Taiwan. *Eur J Clin Microbiol Infect Dis* 1997;16:568-74.

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33. Sanders JW, Martin JW, Hooke M, Hooke J. *Methylobacterium mesophilicum* infection: case report and literature review of an unusual opportunistic pathogen. *Clin Infect Dis* 2000;30:936-8.
34. Alnor D, Frimodt-Moller N, Espersen F, Frederiksen W. Infections with the unusual human pathogens *Agrobacterium* species and *Ochrobactrum anthropi*. *Clin Infect Dis* 1994;18:914-20.
35. Coenye T, Vandamme P, LiPuma JJ. Infection by *Ralstonia* species in cystic fibrosis patients: identification of *R. pickettii* and *R. mannitolilytica* by polymerase chain reaction. *Emerg Infect Dis* 2002;8:692-6.
36. Lewis L, Stock F, Williams D, Weir S, Gill VJ. Infections with *Roseomonas gilardii* and review of characteristics used for biochemical identification and molecular typing. *Am J Clin Pathol* 1997;108:210-6.
37. Brink AJ, van Straten A, van Rensburg AJ. *Shewanella* (*Pseudomonas*) *putrefaciens* bacteremia. *Clin Infect Dis* 1995;20:1327-32.
38. Advisory Committee on Dangerous Pathogens 2004 Approved List of Biological Agents. <http://www.hse.gov.uk/pubns/misc208.pdf>. p. 1-17.
39. Public Health Laboratory Service Standing Advisory Committee on Laboratory Safety. *Safety Precautions: Notes for Guidance*. 4<sup>th</sup> ed. London: Public Health Laboratory Service (PHLS); 1993.
40. *Control of Substances Hazardous to Health Regulations 2002*. General COSHH. Approved Code of Practice and Guidance, L5. Suffolk: HSE Books; 2002.
41. Health and Safety Executive. *5 steps to risk assessment: a step by step guide to a safer and healthier workplace*, IND (G) 163 (REVL). Suffolk: HSE Books; 2002.
42. Health and Safety Executive. *A guide to risk assessment requirements: common provisions in health and safety law*, IND (G) 218 (L). Suffolk: HSE Books; 2002.
43. Health Services Advisory Committee. *Safety in Health Service laboratories. Safe working and the prevention of infection in clinical laboratories and similar facilities*. 2<sup>nd</sup> ed. Suffolk: HSE Books; 2003.
44. NHS Estates. *Health Building Note 15. Accommodation for pathology services*. 1<sup>st</sup> ed. London: Her Majesty's Stationary Office (HMSO); 1991. (Out of print - 2nd edition in press).
45. BS EN 12469: 2000. *Biotechnology - performance criteria for microbiological safety cabinets*. London: British Standards Institution (BSI); 2000.
46. BS 5726: 1992. *Microbiological safety cabinets. Part 2. Recommendations for information to be exchanged between purchaser, vendor and installer and recommendations for installation*. London: British Standards Institution (BSI); 1992.
47. BS 5726: 1992. *Microbiological safety cabinets. Part 4. Recommendations for selection, use and maintenance*. London: British Standards Institution (BSI); 1992.
48. Advisory Committee on Dangerous Pathogens. *The management, design and operation of microbiological containment laboratories*. Suffolk: HSE Books; 2001.
49. Health Protection Agency. *Laboratory Reporting to the Health Protection Agency. Guide for diagnostic laboratories*. February. 2007.

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