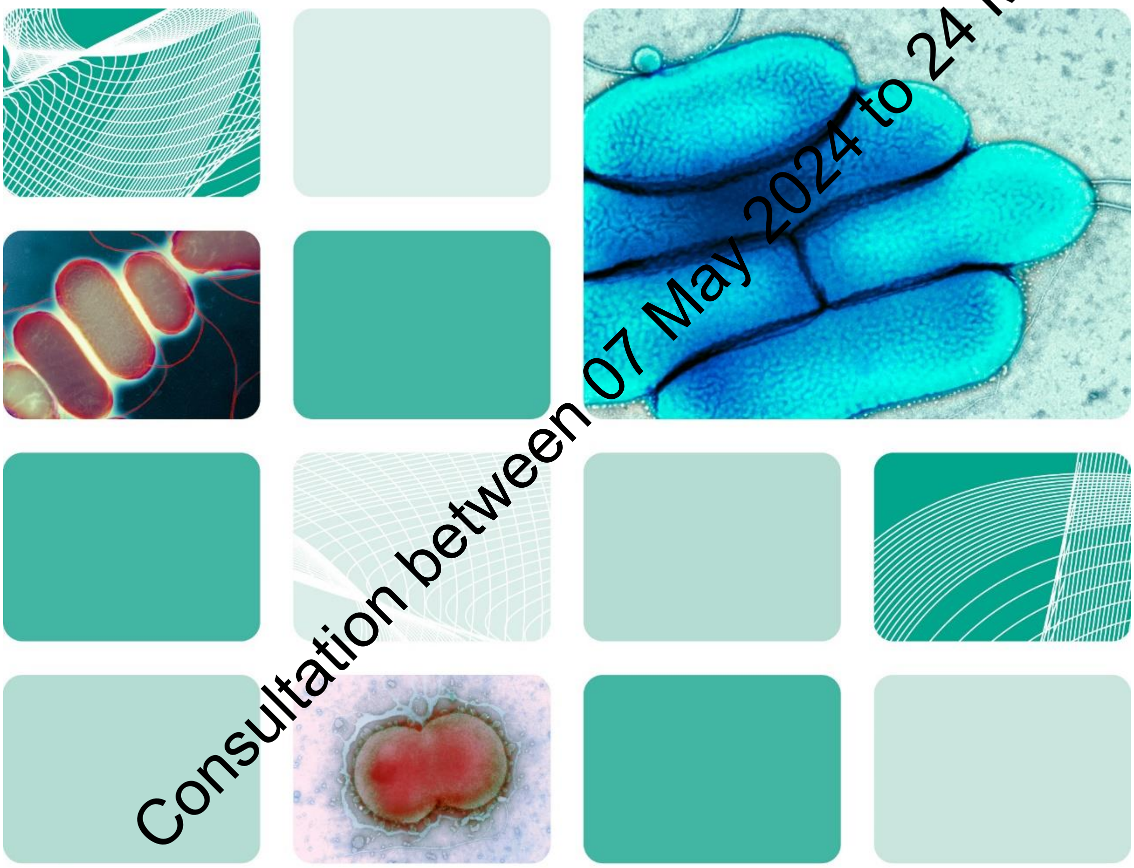
UK Health Security Agency

UK Standards for Microbiology Investigations

Identification of Pseudomonas species and other non-glucose fermenters



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Issued by the Standards Unit, Specialised Microbiology and Laboratories, UKHSA

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Contents

Amendment table

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

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

Amendment number/date	x/dd.mm.yy
Issue number discarded	
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Anticipated next review date*	dd.mm.yy
Section(s) involved	**Amendment**

*Reviews can be extended up to 5 years where appropriate

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

This UK Standards for Microbiology Investigations (UK SMI) document describes the identification of *Pseudomonas* species and other non-glucose fermenters that have been associated with human infection. It includes culture, Gram stain and matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for the identification of microorganisms from culture. Some biochemical tests may not be done routinely in laboratory except in cases where confirmation by an alternative technique is required or automated methods are not available.

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

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

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

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

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

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

4 Introduction

4.1 Taxonomy and characteristics

Pseudomonas species

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

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

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

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

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

Other non-glucose fermenters

Burkholderia species

The genus *Burkholderia* belongs to the family Burkholderiaceae (1) . Many Burkholderia species were reclassified from the genus *Pseudomonas.* The type species is *Burkholderia cepacia* (1)*.* The *B. cepacia* complex (Bcc) consists of 22 closely related species most of which are opportunistic pathogens (11). The species within the *B. cepacia* complex that can cause infections in CF patients are *Burkholderia multivorans* and *Burkholderia cenocepacia,* (12,13). There are a further 3 Burkholderia species associated with human infection, including *Burkholderia gladioli, Burkholderia pseudomallei* and *Burkholderia mallei* (14)*.*

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

Stenotrophomonas maltophilia

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

Acinetobacter species

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

Acinetobacter species are short, non-spore forming Gram negative rods, typically 0.9 - 1.6 by 1.5-2.5µm, often becoming coccoid and appearing as diplococci (18). They may not readily decolourise on Gram staining and demonstrate variable stain retention, along with pleomorphic variations in cell size and arrangement. Many strains are encapsulated. Acinetobacter species are strict aerobes, oxidase negative, catalase positive, non-motile and non-fermentative (18).

Less common non-glucose fermenters

There are many other morphologically similar organisms that have occasionally been isolated from clinical specimens (see Table 1 for details). These organisms are rarely isolated but should be considered. They are usually found in association with contaminated medical devices or in patients who are known to be immuno-compromised. Several of these genera are emerging as nosocomial pathogens and have been linked to outbreaks in cystic fibrosis centres (19,20).

Table 1: Less common non-glucose fermenters (21-34)

Genera	Type Species	Family	Morphology
Acidovorax	*Acidovorax facilis*	*Comamonadaceae*	Straight/Slightly curved rods with one/two polar flagella. Round colonies with smooth to scalloped/spreading margins.
Achromobacter	*Achromobacter xylosoxidans*	*Alcaligenaceae*	Straight rods with rounded ends with 1-20 sheathed flagella. Circular, convex, glistening colonies with smooth margins.
Alcaligenes	*Alcaligenes faecalis*	*Alcaligenaceae*	Rods or coccobacilli with 1-9 peritrichous flagella.
Brevundimonas	*Brevundimonas diminuta*	*Caulobacteraceae*	Rod-shaped, sub-vibrioid, vibrioid with single polar flagella.
Delftia	*Delftia acidovorans*	*Comamonadaceae*	Straight/slightly curved with polar/bipolar tufts of 1-5 flagella.
Elizabethkingia	*Elizabethkingia mengioseptica*	*Weeksellaceae*	Straight, single rods with rounded edges. Circular, convex, shiny colonies.
Comamonas	*Comamonas terrigena*	*Comamonadaceae*	Straight, slightly curved rods/spirilla with polar/bipolar tufts of 1-5 flagella. Colonies are round, convex with smooth to wavy margin, with smooth to granular surface.
Methylobacterium	*Methylobacterium organophilum*	*Methylobacteriaceae*	Rods that occasionally branch/exhibit polar growth with one or more polar flagella. Smooth raised colonies with entire margins.
Ochrobactrum	*Ochrobactrum anthropi*	*Brucellaceae*	Rods with parallel sides/rounded edges and peritrichous flagella. Smooth, low and convex colonies.

Genera	Type Species	Family	Morphology
Pandoraea	*Pandoraea apista*	*Burkholderiaceae*	Rods with one polar flagella.
Ralstonia	*Ralstonia pickettii*	*Burkholderiaceae*	Rods, with single polar flagellum and peritrichous flagella. Beige coloured, domed, smooth colonies.
Roseomonas	*Roseomonas gilardii*	*Acetobacteraceae*	Plump cocci, coccobacilli, short rods. Raised, entire, glistening, mucoid colonies.
Shewanella	*Shewanella putrefaciens*	*Shewanellaceae*	Straight or curved rods with a polar flagellum. Colonies are pale tan to pink-orange.
Sphingobacterium	*Sphingobacterium spiritivorum*	*Sphingobacteriaceae*	Straight rods without flagella.

5 Technical information and limitations

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

Differentiation of species within the *B. cepacia* complex (Bcc) can be particularly problematic, even with an extended panel of biochemical tests, as they are phenotypically very similar and most commercial bacterial identification systems cannot reliably distinguish between them (35). Other organisms such as *S. maltophilia* may be misidentified as Bcc.

6 Safety considerations

The section covers specific safety considerations (36-57) related to this UK SMI, and should be read in conjunction with the general safety considerations.

B. mallei and *B. pseudomallei* are **Hazard Group 3** organisms. Any suspected isolates and specimens must be handled in a containment level 3 room. If these isolates are submitted to the reference laboratory, please contact them in advance. Due to the severity of the risks associated with aerosol exposure, all suspected *B. mallei* and *B. pseudomallei* should be undertaken in a safety cabinet until it has been ruled out.

Laboratory-acquired infections have been reported sporadically among laboratory workers and further potential exposures have been reported (58,59). If exposed, workers should follow local protocols and may be required to start post exposure prophylaxis.

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

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

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

7 Target organisms

Please refer to Table 2 for Pseudomonas species and non-glucose fermenters associated with human disease.

8 Identification

Colonies on primary isolation media are presumptively identified by colonial morphology, Gram stain, oxidase activity and pigment production. Further identification is determined using MALDI-TOF MS or molecular methods including NAAT and 16S rRNA gene sequencing. When MALDI-TOF MS is unavailable, identification can be confirmed using phenotypic tests and/or referral to a suitable reference or specialist laboratory

8.1 Culture methods

Culture methods can be used to provide a presumptive identification of Pseudomonas species and other non-glucose fermenters. Following presumptive identification, further identification methods including MALDI TOF-MS, biochemical tests or molecular methods are used.

8.1.1 Bacterial growth medium

Pseudomonas species have no specific nutritional requirements and are non-fastidious and therefore grow well on all standard laboratory media. Other non-glucose fermenters also grow on most standard laboratory media but may require selective media to inhibit the growth of Pseudomonas species.

Primary isolation media

Isolates will grow on all standard culture media after incubation for 48-72 hours including 5% sheep blood agar in air, Chocolate blood agar in 5-10% CO_2 or MacConkey / CLED agar in air at 35-37°C (5,14). Some strains of *S. maltophilia* may grow best at lower temperatures (60).

For Burkholderia species, selective media is recommended, see section 8.1.1.2

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

Selective media

Selective media can be used to aid in isolation and identification of Pseudomonas species and other non-glucose fermenters. Pseudomonas selective agar can be used when isolating *P. aeruginosa.* Samples should be incubated in air at 35-37°C for 24-72 hours (5,35). It is important to note that on Cetrimide agar medium *P. aeruginosa* can lose its fluorescence under UV if the cultures are left at room temperature for a short time.

Burkholderia selective agar is commercially available and is recommended for isolation of *B. cepacia* complex and *B. pseudomallei* (35,61)*.* For isolation using *B. cepacia* selective agar, isolates should be incubated according to manufacturer's instructions or for at least 72 hours, however growth can take as long as 5 days.

S. maltophilia has been effectively isolated using selective medium with added vancomycin, imipenem, and amphotericin B (14,35).

8.1.2 Colonial appearance

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

8.2 Microscopic appearance

8.2.1 Gram stain

Please refer to UK SMI TP 39 - Staining Procedures.

All species included in this document are Gram negative straight or slightly curved rods. Some Acinetobacter species may not decolourise with Gram staining and have variable stain retention (18). Acinetobacter species can be rod-shaped or cocci (62).

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

Table 2: Microscopic and Colonial appearance of Pseudomonas species and other non-glucose fermenters (3,5,14,15,18,62,63)

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

Species	Appearance	Additional Comments
P. aeruginosa	Small rods with a single polar flagellum. Colonies are usually large and smooth with flat edges but variants that are rough or mucoid exist. Pitting is common larger colonies. Colonies may have a metallic sheen and will fluoresce under short wavelength ultraviolet light.	Can grow in temperatures up to 42°C.
P. putida and *P. fluorescens*	Both do not possess distinctive colony morphology. Both fluoresce under short wavelength ultraviolet light.	Can grow at lower temperatures from 25 °C.
P. monteilii, P. veronii and *P. mosselii*	Motile rods. Colonies are circular and non-pigmented when grown on nutrient agar. They are also non-haemolytic on blood agar.	None
P. stutzeri	Small, motile rods with a singular polar flagellum Colonies are adherent and wrinkled. They can also pit or adhere to the agar and are dark brown. Colonies can become smooth and pale following inoculation.	None
P. mendocina	Colonies are smooth, non-wrinkled and flat producing a brownish yellow pigment.	Can grow at temperatures up to 41°C.
P. alcaligenes and *P. pseudoalcaligenes*	Both species are motile by a singular polar flagellum. They are both non-pigmented and do not have a distinct colony morphology.	Rarely encountered. The optimum growth temperature is 35°C.
P. luteola and *P. oryzihabitans*	Rods with rounded ends and multitricous flagella. Colonies typically exhibit rough, wrinkled and adherent or, more	Growth can occur at 42°C

Species	Appearance	Additional Comments
	rarely smooth colonies. They produce a non-diffusible yellow pigment.	
B. cepacia complex	On MacConkey agar colonies are punctate, tenacous and appear dark pink/red. On blood agar colonies are smooth, raised and occasionally mucoid.	None
B. mallei	On nutrient agar, colonies are smooth, grey and translucent. On MacConkey agar, growth is variable.	Non-motile. Rarely isolated from humans.
B. pseudomallei	Appear as Gram negative bacilli. Colonies are small, smooth and creamy initially. After 48 hours colonies can become wrinkled and dry.	Motile and occasionally can produce a yellow pigment.
S. maltophilia	Straight or curved rods that grow singly or in pairs. Motile with single flagella. Colonies are rough and may appear yellow or green on blood agar.	Some strains can produce slight β-haemolysis.
Acinetobacter species	Plump rods whilst growing but can become spherical during the stationary phase of growth. They often occur in pairs. Colonies are normally smooth, sometimes mucoid, usually non-pigmented.	No flagella but twitching mobility can sometimes be observed.

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

MALDI-TOF MS is used as the primary method for identification of Pseudomonas species and other non-glucose fermenters (64). Therefore, it is important that this method is appropriately validated, manufacturer instructions carefully followed, available database updates installed and reviewed, and the use of an extraction step that can contribute to a more reliable species identification should be considered.

MALDI-TOF MS is used for the identification of Pseudomonas species including *P. aeruginosa* and to identify *B. pseudomallei* (65). This technique has also been utilized to aid in the identification of Acinetobacter species and *S. maltophilia* (66,67).

MALDI-TOF MS, however, may not identify specific species within the *B. cepacia* complex (68). In this case, further testing or referral to reference or specialist laboratories is recommended. It is important to note that when results are inconsistent, further methods should be used to ensure accurate identification of isolates.

When less common non-glucose fermenters, including those in Table 1, are suspected, MALDI-TOF MS should be used for identification, when available. MALDI-TOF MS may not be able to identify Achromobacter, Pandorea, Elizabethkingea and Ralstonia species as they are not included in most databases, and therefore molecular methods should be used (35).

8.4 Further Identification

8.4.1 Biochemical tests and commercial identification systems

Biochemical tests are no longer routine in laboratories but are used in cases when MALDI-TOF MS is unavailable or when MALDI-TOF MS results are inconclusive. Discrepancies in test results should be referred to the appropriate reference or specialist laboratories for further testing. Assessing nitrate reduction, arginine dihydrolase and pyoverdine production, for example, can help differentiate between similar species. For example, *P. aeruginosa* have positive arginine dihydrolase, nitrate reduction and oxidase tests, which when coupled with colonial morphology, can provide identification.

Examples of biochemical tests used for identification purposes are included in Algorithm B. For full biochemical profiles of all the species in this document, please consult the relevant chapters in the *Manual for Clinical Microbiology* book or manufacturer's guidance (5,14,62).

Please note biochemical tests are not recommended as an identification method for samples from cystic fibrosis patients (35).

8.4.1.1 Oxidase test

Please refer to UK SMI TP 26 - Oxidase Test.

Most Pseudomonas species are oxidase positive, except *P. luteola* and *P. oryzihabitans. P. aeruginosa* is oxidase positive however, some strains of *P. aeruginosa* display a slow oxidase reaction (5).

Other non-glucose fermenters have variable results depending on species. *B. pseudomallei* are oxidase positive, whereas *B. mallei* and *B. cepacia* complex have variable results to the oxidase test. When coupled with colonial morphology, the results of the oxidase test can aid in providing preliminary identification for isolates (14).

8.4.1.2 Commercial identification systems

Commercial identification systems do not provide definitive speciation of many of the clinically significant, glucose non-fermenting Gram negative bacilli. There are some that are available for identification of *P. aeruginosa* however for identification of other Pseudomonas species, commercial identification kits are not considered accurate (5). Commercial identification systems can be used to identify species in the *Burkholderia cepacia complex* but are not sufficient alone and should be confirmed using molecular methods (69).

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

8.4.2 Molecular Methods

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

NAATs have been assessed for the rapid and reliable identification of *P. aeruginosa.* NAATs are much faster than culture-based methods and could allow cystic fibrosis patients to receive treatment quickly (70).

They have been used successfully for the rapid and reliable detection of pathogenic Burkholderia species – *Burkholderia mallei* and *Burkholderia pseudomallei*. The high reliability and sensitivity 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 (71).

NAAT of the recA gene or Whole Genome Sequencing are recommended when organisms in the Bcc are suspected in cystic fibrosis patients, to allow accurate speciation. If unavailable in the diagnostic laboratory, isolates should be sent to the relevant reference laboratory (35).

9 Storage and Transport

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

For long term storage, isolates should be frozen at -80°C (72).

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

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

Follow instructions provided by the reference laboratory for sending isolates. Refer to Section 11: Referral to reference laboratories for sending isolates to the appropriate laboratory

10 Reporting

10.1 Infection Specialist

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

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

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

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

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

Follow local protocols for reporting to clinician.

10.2 Routine identification

Appropriate growth characteristics, colonial appearance, Gram stain and oxidase results can give an early indication of the isolation of *Pseudomonas* or other non-fermenting gram-negative isolates however, full identification by MALDI-TOF MS should be achieved. If MALDI-TOF MS is not available confirmation of the identification is required.

10.3 Confirmation of identification

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

10.4 Health Protection Team (HPT)

Refer to local agreements in devolved administrations.

10.5 UK Health Security Agency

Refer to current guidelines on Second Generation Surveillance System (SGSS) reporting (52).

10.6 Infection prevention and control team

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

11 Referral to reference or specialist laboratories

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

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

England

Wales

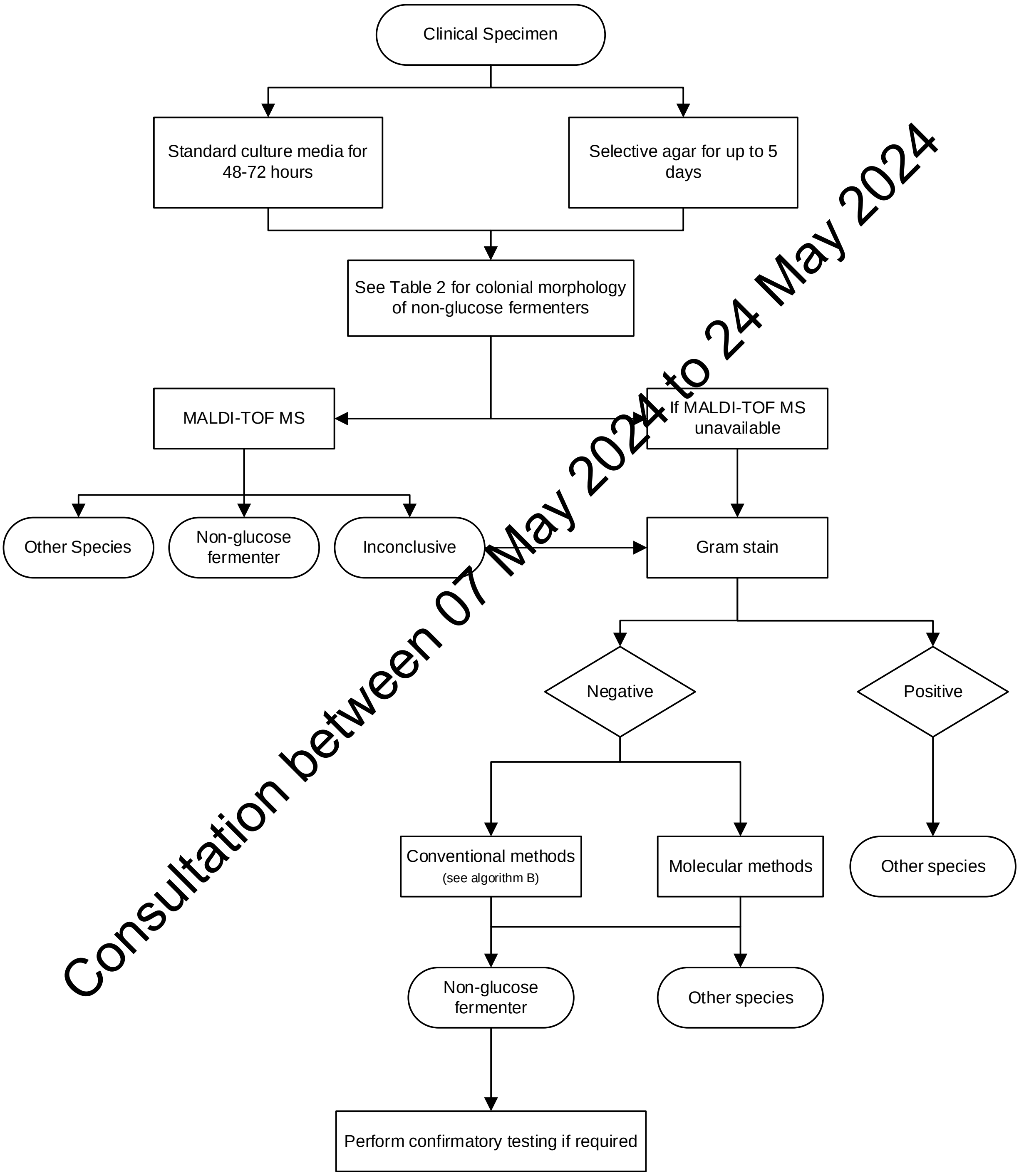
Scotland

Northern Ireland

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

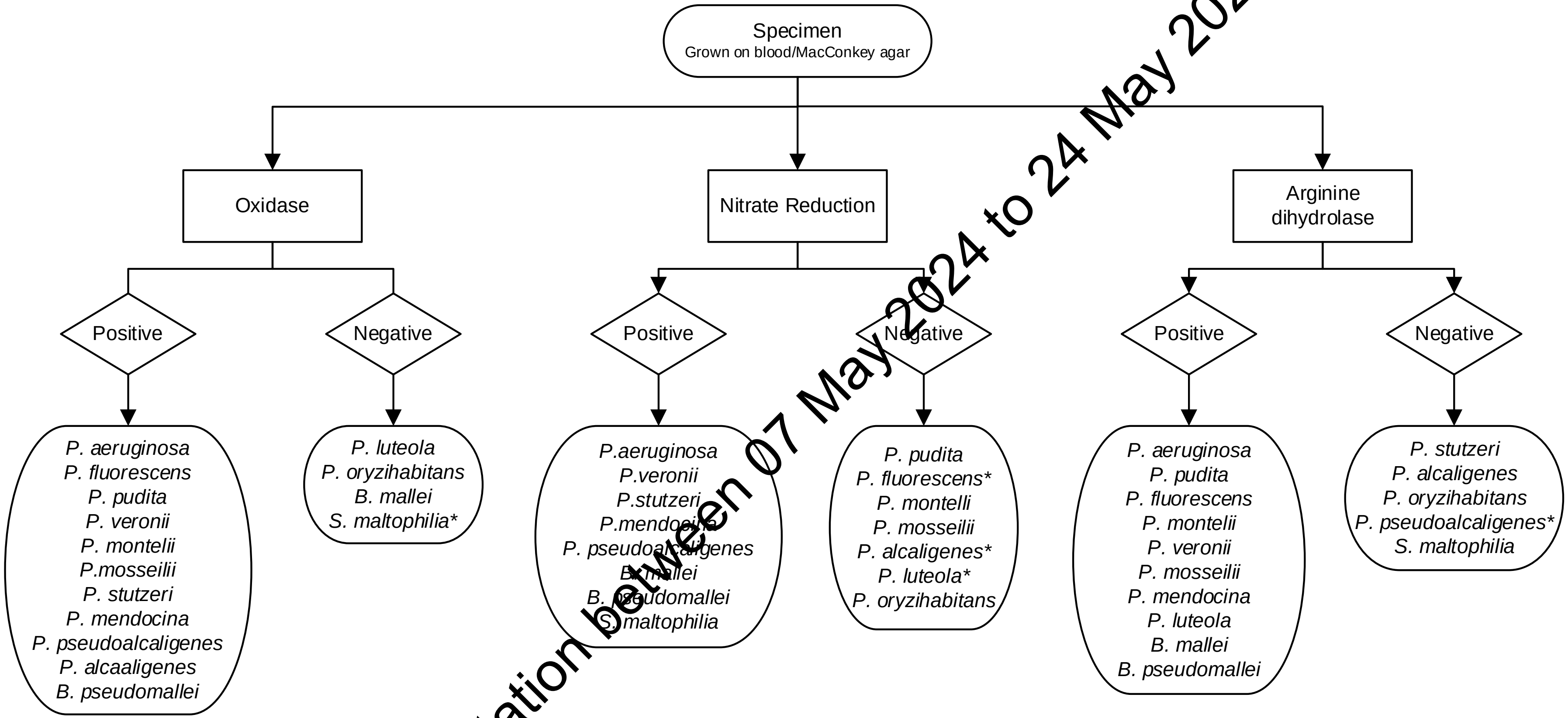
Algorithm A: Identification of non-glucose fermenters

This flowchart is for guidance only.



Algorithm B: Further identification of non-glucose fermenters

This flowchart provides a summary of biochemical tests to supplement algorithm A. This flowchart is for guidance only.



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An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.

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