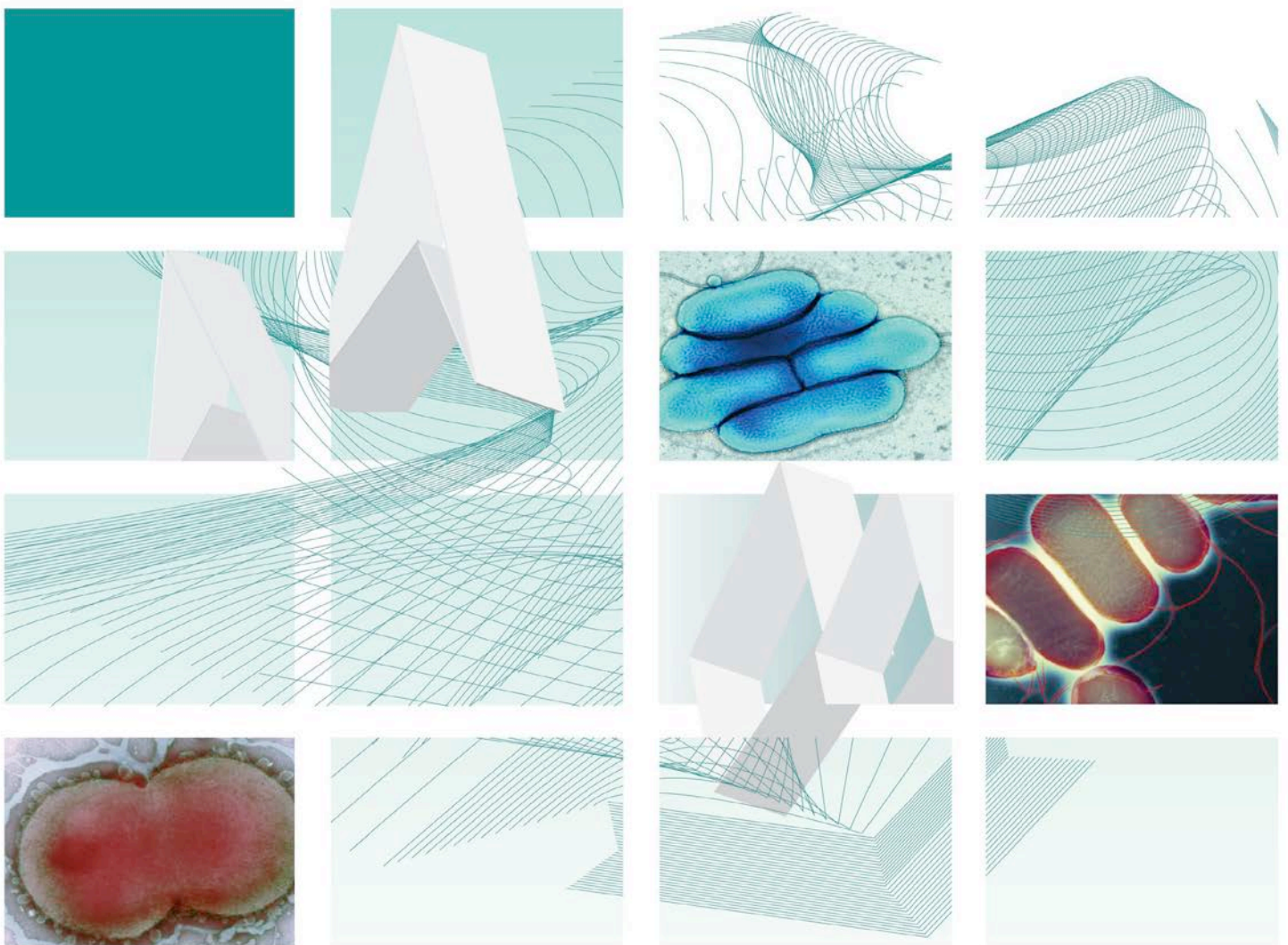




# UK Standards for Microbiology Investigations

## Identification of *Moraxella* species and Morphologically Similar Organisms



## Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee>).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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UK Standards for Microbiology Investigations are produced in association with:



Logos correct at time of publishing.

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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.	7/03.02.15
Issue no. discarded.	2.4
Insert Issue no.	3
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Whole document.	Document presented in a new format. Reorganisation of some text. Edited for clarity. Test procedures updated. Updated contact detail of Reference Laboratory.
Scope of document.	The scope has been updated to include webpage links for ID 6, ID 12 and ID 17 documents.
Introduction.	The taxonomy of <i>Moraxella</i> species and other similar organisms has been updated. More information has been added to the Characteristics section. The medically important species have been grouped and their characteristics described. Use of up-to-date references. Section on Principles of Identification has been amended for clarity.
Technical Information/Limitations.	Addition of information regarding oxidase test and commercial identification systems has been described and referenced.
Safety considerations.	Reference added. Text re-organised.
Target Organisms.	The section on the Target organisms has been updated and presented clearly. References have

Identification of *Moraxella* species and Morphologically Similar Organisms

	been updated.
Identification.	Amendments and updates have been done on 3.1, 3.2, 3.3 and 3.4 have been updated to reflect standards in practice. Subsection 3.5 has been updated to include the Rapid Molecular Methods.
Identification Flowchart.	Modification of flowchart for identification of species has been done for easy guidance.
Reporting.	Subsections 5.1 and 5.5 has been updated to reflect reporting practice.
Referral.	The contact detail of the reference laboratory has been updated.
References.	Some 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 <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>.

Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process.

SMIs are developed, reviewed and updated through a wide consultation process.

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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

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions.

The development of SMIs are subject to PHE Equality objectives <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>. The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

## Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

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**

Public Health England. (2015). Identification of *Moraxella* species and Morphologically Similar Organisms. UK Standards for Microbiology Investigations. ID 11 Issue 3.

<https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>



## Scope of Document

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This SMI describes the identification of *Moraxella* species and those species which are morphologically similar.

To differentiate *Moraxella* species from *Neisseria* species see [ID 6 – Identification of \*Neisseria\* species](#).

*Acinetobacter* species may also be misidentified as *Moraxella* species and their identification is described in [ID 17 - Identification of \*Pseudomonas\* species and morphologically similar organisms](#).

The *Kingella* species is described in [ID 12 - Identification of \*Haemophilus\* species and the HACEK Group of Organisms](#).

This SMI should be used in conjunction with other SMIs.

## Introduction

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### Taxonomy

The genera *Moraxella* (including the former *Branhamella*), *Acinetobacter*, *Psychrobacter*, *Alkanindiges*, *Enhydrobacter*, *Paraperlucidibaca* and *Perlucidibaca* currently belong to the family Moraxellaceae.

The *Moraxella* genus currently contains 22 different species, including *M. catarrhalis*, *M. bovis*, *M. lacunata*, *M. osloensis*, *M. nonliquefaciens*, *M. atlantae*, *M. lincolni*, *M. ovis*, *M. caviae*, *M. canis*, *M. equi*, *M. cuniculi*, *M. caprae*, *M. anatipestifer*, *M. bovoculi*, *M. oblonga*, *M. phenylpyruvica*, *M. pluranimalium*, *M. porci*, *M. saccharolytica*, *M. urethralis* and *M. boevrei*, which colonize both humans and animals<sup>1</sup>. The genus is under constant revision, with recent taxonomic restructuring placing the bacterial species in different genera, eg *Moraxella phenylpyruvica* as formerly known has been moved into the genus *Psychrobacter* as *Psychrobacter phenylpyruvica* and *Moraxella urethralis* in the *Oligella* genus as *Oligella urethralis*. *M. anatipestifer* has also been reclassified to the genus *Riemerella* as *Riemerella anatipestifer*.

### Characteristics

#### Genus *Moraxella*<sup>2,3</sup>

*Moraxella* species are Gram negative rods or cocci, but often with a tendency to resist decolourisation. The rods are often very short and plump, approaching a coccus shape 1.0 - 1.5 x 1.5 - 2.5µm. Cells usually occur in pairs or short chains with one plane of division. Pleomorphism is enhanced by lack of oxygen and by incubation at temperatures above the optimum. The medically important species (rod-shaped) are *M. atlantae*, *M. lacunata*, *M. nonliquefaciens* and *M. osloensis*.

The cocci are usually smaller (0.6 - 1.0µm in diameter) and occur singly or in pairs with adjacent sides flattened, and sometimes tetrads are formed. There is one medically important species, *M. catarrhalis*.

Cells may be capsulated. They are non-motile and aerobic, but some strains may grow weakly under anaerobic conditions. Most species except *Moraxella osloensis* are nutritionally fastidious and growth on standard media may be poor or fail; some are

stimulated significantly by fatty acids (bile salts, Tween 80). The optimum growth temperature is 33 - 35°C. *Moraxella* species are usually catalase and oxidase positive and do not produce acid from carbohydrates. Nitrate may or may not be reduced.

Common characteristics of the *Moraxella* genus include a lack of colony pigmentation; Gram negative staining coccobacillus and *Bacillus* morphology (except *M. catarrhalis*, which exhibits a coccoid morphology).

*Moraxella* species have been isolated from the conjunctiva, upper respiratory tract, blood, inflammatory secretions of the middle ear, maxillary sinus, bronchial aspirate, nasal cavity, spleen, cerebrospinal fluid, genitourethral tract, joints and bursa of humans.

**The medically important *Moraxella* species are:**

***M. atlantae*<sup>4</sup>**

They are variably sized, often plump, diplococcobacillary to distinctly rod-shaped cells (average 1.0 x 2.0µm), with little tendency to grow in longer chains and to resist decolourisation. They are often fimbriated and not encapsulated. They are also not pigmented and are non-motile. The optimal growth temperatures are 33 - 37°C and are strictly aerobic.

Colonies are usually small, non-haemolytic, slightly opaque, 0.5mm in diameter and show spreading and pitting of the agar. Two main colony variants occur, one hemispherical with an even outline, the other more flat or with irregular margin, and with a tendency to form a spreading zone. Pitting of the agar, more pronounced beneath the latter colony variant.

They are positive for oxidase and catalase tests but are negative for acid production from carbohydrates, nitrate and nitrite reduction, urease, indole and H<sub>2</sub>S production.

*M. atlantae* have been isolated from human blood, cerebrospinal fluid and spleen.

***M. lacunata*<sup>5</sup>**

Cells are medium thick to plump rods, 0.8 - 1.2µm in diameter, occurring in diploid pairs and chains. They have the tendency to lose their Gram negative staining characteristics when left out for days and to retain these new characteristics on subsequent blood agar transfers. They are frequently pleomorphic and may form narrow capsules. Colonies are small (0.1 - 0.3mm in diameter), translucent to semi-opaque, form dark haloes on chocolate agar and pitting of the agar is common. On blood agar, no haemolysis is observed. Some strains of *M. lacunata* are haemolytic.

They are positive for oxidase and catalase tests and negative for indole production.

*M. lacunata* has been isolated from the human eye and from knee infections (synovial fluid)<sup>6</sup>.

***M. nonliquefaciens*<sup>7</sup>**

Cells are plump rods with obtuse, often nearly square ends, often very short diplobacilli, occasionally occurring in short chains. Diplococcus-like forms are frequent. They may be encapsulated and have no endospores. They are strictly aerobic and optimal growth is at 33 - 37°C. Colonies of *M. nonliquefaciens* are small (0.5 - 1mm in diameter), low convex or nearly flat, smooth, translucent to semi-opaque on blood agar after 24hr and the colonies will occasionally spread and pit the agar. They are unpigmented, non-haemolytic and have a soft or friable consistency. Some strains are strongly mucoid with large, domed, shiny and viscous colonies.

They are positive for catalase and oxidase tests, nitrate reduction; and negative for acid production from carbohydrates, gelatin liquefaction, indole and H<sub>2</sub>S production. Some strains split urea immediately after isolation, but this property is lost in subculture.

They have been isolated from the human respiratory tract but most frequently the nose.

### ***M. lincolnii***<sup>8</sup>

Cells are coccus-like to plump rods 1 - 1.5µm wide and 1.5 - 2.5µm long. The cells often occur in pairs and may form short chains. After 2 days of incubation, colonies are whitish, smooth, convex, and circular and have a diameter of 1 to 3mm. The colonies of some strains may have a flattened edge. There is no haemolysis and no production of pigment or odour. They grow under aerobic, capnophilic, or microaerobic conditions but not anaerobically. They grow on blood agar or nutrient agar. Optimal growth occurs at 28 to 33°C. Growth also occurs at 36 - 37°C, but not at 42°C. Growth occurs in the absence of NaCl and they are positive for oxidase and catalase activities. Most strains reduce nitrites. No fermentation or oxidation of D-glucose.

They are negative for acid production from D-glucose, maltose, D-fructose, or sucrose; urease, DNase, or β-galactosidase activity, nitrate reduction, liquefaction of gelatin, proteolysis on Loeffler slants, hydrolysis of Tween 80, or indole production. All strains are susceptible to penicillin (10µg discs).

*M. lincolnii* has been isolated mainly from the respiratory tract of humans.

### ***M. osloensis***<sup>7</sup>

Cells are like *M. nonliquefaciens* although some strains show a more fusiform or lanceolate shape, others show a preponderance of diplococcal cells. They are non-motile, non-spore-producing, non-encapsulated. They are strictly aerobic and optimal growth is at 33 - 37°C. Colonies of *M. osloensis* and *M. lincolnii* are similar in appearance, but pitting of the agar is rare and they have a soft or coherent consistency and are unpigmented. Nitrates may or may not be reduced to nitrites. They are negative for urease activity except for irregular reactions which may be observed in fresh isolates.

Strains have been isolated from genito-urinary tract, blood, spinal fluid, chest fluid, and nose, but seem to be rare in respiratory tract.

### ***M. catarrhalis*** (Previously known as ***Branhamella catarrhalis***)<sup>9</sup>

*M. catarrhalis* appears as extracellular, kidney-shaped diplococci, measuring 0.5 - 1.5µm in diameter on Gram stained clinical specimens. They grow well on blood agar as well as chocolate agar but not on MacConkey agar. On blood agar, colonies are non-haemolytic, grey to white, opaque, smooth, dry, and 1 - 3mm in diameter after 24hr incubation and on chocolate agar; colonies are pinkish brown, resembling *Neisseria gonorrhoeae* colonies. Colonies remain intact when pushed across the surface of the agar and are unpigmented.

*Moraxella catarrhalis* is the most frequently isolated species of *Moraxella* and can be differentiated from *Neisseria* species by the tributyrin test: *M. catarrhalis* is positive and *Neisseria* species are negative<sup>10-12</sup>. However, as the tributyrin test is positive for *Moraxella* species other than *M. catarrhalis*, it cannot be used alone to differentiate among the *Moraxella* species<sup>10-12</sup>.

They are positive for oxidase test, DNase production, and reduction of nitrates to nitrites and negative to failure to produce acid from glucose, maltose, sucrose, lactose, and fructose.

Most strains of *M. catarrhalis* are  $\beta$ -lactamase positive<sup>9</sup>.

It has been isolated from nasopharynx, throat, ear effusions and sinus aspirates<sup>13</sup>.

### **Other Morphologically Similar Organisms are:**

#### ***Oligella* species<sup>2</sup>**

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

*Oligella* species are oxidase positive and usually catalase positive and neither ferment or oxidise carbohydrates.

They are mainly isolated from the genitourinary tract of humans.

There are currently only 2 species in this genus; *O. ureolytica* (previously known as CDC group IVe) and *O. urethralis*<sup>14</sup>.

#### ***O. ureolytica***

Previously known as CDC group IVe. They do not grow at 42°C and 31 - 79% of strains are motile by means of long peritrichous flagella. *Oligella ureolytica* grows slowly on blood agar producing pinpoint colonies after 24hrs 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.

*Oligella urethralis* can also grow in the presence of 3% NaCl and are positive for urease test. Some strains are positive for nitrate reduction as well as denitrification. They also utilize *p*-hydroxybenzoate as a carbon source for growth. Isolated from human urine<sup>15</sup>.

#### ***O. urethralis***

Previously classified as *Moraxella urethralis*. They are non-motile and grow at 42°C. They can also grow in the presence of 3% NaCl and are negative for nitrate reduction and urease test. They do not utilize *p*-hydroxybenzoate as a carbon source for growth. *O. urethralis* has been isolated from urine, the urinary tract, and also the ear<sup>15</sup>.

#### ***Kingella* species**

There are currently 4 valid species in this genus: *K. denitrificans*, *K. kingae*, *K. oralis* and *K. potus*<sup>16</sup>. *K. indologenes* has been reclassified to the genus *Suttonella* as *Suttonella indologenes*<sup>17</sup>.

*Kingella* species are straight rods, 1.0 $\mu$ m in length with rounded or square ends. They occur in pairs and sometimes short chains. Endospores are not formed. Cells are Gram negative, but tend to resist decolourization. Two types of colonies occur on blood agar; a spreading, corroding type and a smooth, convex type. It does not

require X or V factors. Growth is aerobic or facultatively anaerobic. The optimum growth temperature is 33 - 37°C<sup>2</sup>.

They are non-motile, oxidase positive, catalase negative and urease negative. Glucose and other carbohydrates are fermented with the production of acid but not gas.

*Kingella* species may grow on *Neisseria* selective agar and therefore may be misidentified as pathogenic *Neisseria* species. They can be differentiated from *Moraxella* and *Neisseria* species by a catalase test. Most *Kingella* species are catalase negative; *Moraxella* and most *Neisseria* species (except *Neisseria elongata*) are catalase positive.

### ***K. denitrificans***<sup>18</sup>

Previously designated CDC group TM-1. They are plump rods 1.0µm in width. Small, translucent non-haemolytic colonies are produced on blood agar after 48hr of incubation at 37°C. Colonies may show pitting of the medium. Growth occurs anaerobically on blood agar. They are positive for oxidase, growth at 30 and 37°C, fermentative result in the O/F test, acid production from glucose, nitrate reduction, nitrite reduction, and production of gas from nitrite.

They are also negative for catalase, growth at 5 and 45°C, growth in the presence of 4 and 6% NaCl, growth on β-hydroxybutyrate in mineral medium, acid production from maltose unless serum was present, starch hydrolysis and urease production. Isolated in the respiratory tract of man<sup>19</sup>.

### ***K. kingae***<sup>20</sup>

The cells are coccoid to medium-sized rods, very much like those of *Moraxella* but slightly smaller, have square ends, and occur in pairs and short chains. They are Gram negative, with some tendency to resist decolourisation. They are also non-motile, non-encapsulated and no endospores are produced. On blood agar, two types of colonies occur; colonies of freshly isolated strains appear as small depressions, 0.1 - 0.5mm in diameter, with a small central papilla initially but after 2 or more days incubation, there is considerable spreading growth and thin granular zones of growth often surround the colonies. Colonies when scraped shows corrosion marks on the agar surface. The second colony, which often arises in subcultures of the first type, is small, delicate, translucent or slightly opaque, 0.1 - 0.6mm in diameter after 20hr on blood agar, low hemispherical, and smooth. On further incubation, the colonies increase in size but there is no evidence of corrosion or spreading. Both types of colonies are surrounded by distinct zones of β-haemolysis; their consistencies are soft or coherent and are not pigmented.

They are aerobic and grow at room temperature but their optimal growth is at 33 - 37°C. They are relatively fastidious and growth on high quality nutrient agar is as good as that on blood agar.

*K. kingae* are negative for catalase and urease tests. No acid is produced from fructose, lactose, saccharose, arabinose, xylose, rhamnose, mannitol, dulcitol, sorbitol, or glycerol. Gelatin and serum are not liquefied. Nitrates are either not reduced or slightly reduced.

They are parasitic on human mucous membranes. Strains have been isolated from throat, nose, blood, bone lesions and joints.

### ***K. oralis***<sup>21</sup>

They are rods or coccobacilli approximately 0.6 - 0.7µm in diameter by 1 - 3µm long with rounded ends. Cells can form pairs or chains. Cells have monopolar fimbriae up to 10µm long. There is a tendency to resist Gram decolourisation. Not motile by means of flagella, but cells form spreading colonies. They are aerobic or facultatively anaerobic. Growth is supported by 5% sheep blood agar supplemented with 5mg of haemin per litre and 0.5µg of menadione per mL in both anaerobic and aerobic environments with CO<sub>2</sub>. They do not grow on MacConkey agar. Colonies are round with slightly irregular borders and flat to umbonate, and each colony has a granular periphery. Colonies appear to corrode the agar surface. They are positive for oxidase test and negative for nitrate, nitrite, indole, urease and aesculin hydrolysis tests. Acid is not produced from lactose, maltose, mannitol, sucrose, and xylose.

The habitat of *K. oralis* appears to be human dental plaque and has been isolated from a supragingival plaque sample from a patient with adult periodontitis.

### ***K. potus***<sup>22</sup>

They are aerobic, DNase positive, oxidase positive, and catalase negative. Colonies are circular, low convex, yellow- pigmented, smooth, entire, approximately 1.5 - 2mm in diameter, and friable on Columbia blood agar after 48hr of incubation at 37°C. Colonies are non-haemolytic. Non-diffusible yellow pigments are produced. Nitrate and nitrite are not reduced. Aesculin and urea are not hydrolysed. Indole is not produced. Acid is not produced from fructose, glucose, mannose, mannitol, maltose, lactose, or sucrose. No alkaline phosphatase, α-glycosidase, β-galactosidase, or β-glucuronidase activity is detected. This has been isolated from a human wound caused by a bite from a kinkajou.

Tests that are useful in distinguishing *Kingella potus* from other *Kingella* species and members of the genus *Neisseria* are DNase test and its ability to pigment.

### ***Psychrobacter* species**<sup>5,23</sup>

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

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

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



There are currently 34 valid species and 6 of which have been isolated from humans<sup>24</sup>. They are as follows; *P. arenosus*, *P. immobilis*, *P. faecalis*, *P. phenylpyruvicus*, *P. pulmonis* and *P. sanguinis*.

### ***P. arenosus***<sup>25,26</sup>

They are aerobic, non-pigmented, non-spore-forming, ovoid cells (1.4 - 1.7µm long and 0.6 - 0.8µm in diameter). They are psychrotolerant and grow at 4 - 37°C, with an optimum growth temperature of 25 - 28°C. They do not grow at 39 or 40°C. They also grow at pH 5.0–10.0, with optimum growth at pH 6.0– 9.0. Sodium ions are not required for growth; growth occurs in 0–10% (w/v) NaCl, but not in 12% NaCl. On blood agar, colonies are monomorphic, small, and grey and on tryptic soy agar, colonies are opaque, circular, convex, and cream coloured.

Acid is formed from D-glucose, rhamnose, galactose, lactose and arabinose. They are positive for oxidase and catalase tests but negative for urease, indole production, hydrolysis of aesculin and gelatin and utilization of glucose, arabinose, mannose, maltose and mannitol.

*P. arenosus* was originally isolated from a marine sediment sand sample from the Sea of Japan, Russian territorial waters but has been recently isolated from a contaminated erythrocyte unit and blood of a patient undergoing transfusion after patient fell ill.

### ***P. immobilis***<sup>27</sup>

They are plump coccobacilli frequently showing diploforms. They grow at temperatures from 5 - 25°C but fail to grow at 35 - 37°C. Acid is formed aerobically from glucose, mannose, galactose, arabinose, xylose, and rhamnose but is not formed from fructose, maltose, or sucrose. They are positive for nitrate, deamination of phenylalanine and tryptophan and urease test and negative for starch, gelatin, and serum hydrolysis and indole and H<sub>2</sub>S production.

*P. immobilis* has been isolated from sources such as the eye, brain tissue, urethra, cerebral spinal fluid, and blood, leading some scientists to suspect that these bacteria may be the cause of opportunistic infections in some patients. The clinical manifestation of this species is virtually unknown, although it has been isolated in patients with meningitis, AIDS and other infections.

### ***P. faecalis***<sup>28</sup>

Cells are straight rods, 0.8 - 1.2 x 1.0 - 2.0µm. Cells occur singly and are non-motile, Gram negative, oxidase positive and catalase positive, with an oxidative, chemoheterotrophic metabolism. On nutrient agar, colonies are circular, opaque, slightly raised and beige with entire margins. No growth is observed at 45 or 55°C on nutrient agar. They are negative for indole production, urease, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and growth on Simmons' citrate. They are also saccharolytic with acid production from glucose, arabinose, lactose, galactose, melibiose, cellobiose, maltose and xylose but not from mannitol. Acid is also produced from ethylene glycol.

*P. faecalis* and *P. pulmonis* are urease negative and nitrite reductase positive, which easily differentiates them from *P. phenylpyruvicus* and *P. immobilis*.

It has been isolated in clinical specimens from humans, such as wound, nasopharynx, pus, pleural fluid, conjunctival secretions and lymph node.

### ***P. phenylpyruvicus*<sup>29</sup>**

They are coccoid, oxidase positive, non-motile bacteria which are psychrotolerant and halotolerant. The optimal growth temperature is 10°C and do not grow at 30°C or higher. Growth of *P. phenylpyruvicus* is drastically enhanced by addition of 1% Tween 80 to the medium. Cream coloured colonies which were circular, slightly convex, and about 2 - 4mm in diameter appeared on the isolation plates after 3 to 7 days.

They are positive for catalase and oxidase; growth at 4 - 15°C; tolerance to 6.5% NaCl; production of C8 esterase, alanine arylamidase, and leucine arylamidase; hydrolysis of uric acid and Tween 80; and utilization of butyrate, L-asparagine, L-glutamate, and L-proline as sole carbon and energy sources. 90 - 100% of the strains of *P. phenylpyruvicus* are urease positive. They are negative for nitrate reduction, Simmons citrate test and acid production from L-Arabinose, D-xylose, and D-raffinose D-mannose, cellobiose, D-melibiose, and N-acetylglucosamine.

*Brucella* species can be misidentified as *P. phenylpyruvicus* in some commercial identification kits<sup>30</sup>.

*P. phenylpyruvicus* has been isolated from human blood and cerebrospinal fluid.

### ***P. pulmonis*<sup>31</sup>**

They are non-motile, coccus-shaped cells that are catalase and oxidase positive. They are strictly aerobic and on blood agar at 37°C, they form non-pigmented, smooth colonies. Growth does not occur on MacConkey agar.

They are positive for growth in 6.5% NaCl, nitrate reduction, and production of acetoin and are negative to urea, gelatin and aesculin hydrolysis; production of indole or H<sub>2</sub>S and production of acid from glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin or arabinose.

*P. pulmonis* has been isolated from human blood.

### ***P. sanguinis*<sup>32</sup>**

They are non-haemolytic, non-motile, non-pigmented, non-sporulating coccobacilli (0.5 - 1.0µm wide and 1.0 - 2.0µm long). Colonies are 1 - 2mm in diameter, moist, non-pigmented, circular and smooth with entire margins. Grows at 4 - 37°C (optimum is between 30 - 37°C). Growth is observed on marine agar and in marine broth<sup>23</sup>. No growth is observed on MacConkey agar, Trypticase Soy agar, Brain heart infusion agar or Luria-Bertani agar. Cells are positive for oxidase and catalase, have strong urease activity and are able to reduce nitrate to nitrite. Cells are negative for acid production on Hugh and Leifson oxidation-fermentation medium with 1% D-glucose, maltose, D-mannitol, lactose, sucrose and D-xylose. They are also negative for growth at 42°C, utilization of Simmons' citrate, hydrolysis of aesculin and gelatin, production of indole and complete decarboxylation of arginine, lysine, and ornithine in Moeller's decarboxylase medium.

*P. sanguinis*<sup>32</sup> has been isolated from human blood.

## **Principles of Identification**

Colonies isolated on chocolate or blood agar plates are identified by colonial morphology, Gram stain and oxidase reaction. Further biochemical identification may be performed. If required, isolates may be referred to the Reference Laboratory for confirmation and further identification.

## Technical Information/Limitations

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### Oxidase Test

*Kingella* species and *M. catarrhalis* are oxidase positive and can be misidentified as *Neisseria* species.

### Commercial Identification Systems

Commercial kits may misidentify *Brucella* species as *P. phenylpyruvicus*<sup>30</sup>.

## 1 Safety Considerations<sup>33-49</sup>

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Hazard group 2 organisms.

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<sup>41</sup>.

Consider *Neisseria meningitidis* from respiratory samples ie aerosol droplets.

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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***Moraxella* species and morphologically similar organisms reported to have caused human infection**<sup>6,8,13,21,22,28,29,32,50,51</sup>

*M. catarrhalis*, *M. atlantae*, *M. lacunata*, *M. nonliquefaciens*, *M. osloensis*, *M. lincolnii*, *K. denitrificans*, *K. kingae*, *K. oralis*, *K. potus*, *O. urethralis*, *O. ureolytica*, *P. immobilis*, *P. phenylpyruvicus*, *P. faecalis*, *P. pulmonis*, *P. sanguinis*, *P. arenosus*

## 3 Identification

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### 3.1 Microscopic Appearance

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

Gram negative with a tendency to resist decolourisation.

#### ***Moraxella* species**

Rods, often coccobacilli. Usually occur in pairs or short chains with one plane of division. Sometimes, it could appear as cocci occurring singly or in pairs with adjacent sides flattened, forming tetrads.

#### ***Kingella* species**

Plump rods or coccobacilli occurring in pairs or chains.

#### ***Oligella* species**

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

#### ***Psychrobacter* species**

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

### 3.2 Primary Isolation Media

Blood or chocolate agar incubated in 5 - 10% CO<sub>2</sub> at 35°C - 37°C for 16 - 48hr.

### 3.3 Colonial Appearance

#### ***Moraxella* species**

Colonies are smooth, flat, uniform, buff and 1 - 2mm in diameter.

Colonies of *M. lacunata*, *M. atlantae* and *M. liquefaciens* are small <1mm on blood agar. *M. lacunata* and *M. atlantae* may pit the agar. Some strains of *M. lacunata* are haemolytic. Colonies can appear smooth, round, uniform, grey/brown and 1mm in diameter.

On chocolate agar, colonies *M. catarrhalis* are pinkish brown, resembling *N. gonorrhoeae* colonies.

#### ***Kingella* species**

Two types of colonies occur on blood agar, a smooth entire convex type and a spreading colony. Colonies are small, 0.5 - 1mm in diameter after 48hr.

**Note:** *K. kingae* produce distinct zones of beta-haemolysis.

#### ***Oligella* species**

Colonies are small, white, opaque, entire and non-haemolytic after 24hr incubation.

#### ***Psychrobacter* species**

Require incubation at 20°C - 25°C. Colonies are small, smooth and opaque on blood agar. Growth is enhanced by bile salts or Tween 80 to form non-pigmented, smooth, opaque colonies.

### 3.4 Test Procedures

#### 3.4.1 Biochemical tests

##### **Oxidase test ([TP 26 – Oxidase Test](#))**

*Moraxella* species are oxidase positive.

*Neisseria* species are also oxidase positive and may be misidentified as *Moraxella* species.

##### **Tributylin test**

2-4hr Presumptive test

*Moraxella* species are Tributyrin positive.

This test is also used to distinguish *M. catarrhalis* from *Neisseria* species - *M. catarrhalis* is positive and *Neisseria* species are negative<sup>12</sup>.

##### **Supplementary tests**

##### **DNase test ([TP 12 – Deoxyribonuclease Test](#))**

Positive for *M. catarrhalis*.

The DNase test may be used as a supplementary test to differentiate *M. catarrhalis* from other *Moraxella* species.

### 3.4.2 Commercial identification Systems

Other identification systems including commercial kits may be used as a supplementary or confirmation test. Laboratories should follow manufacturer's instructions and rapid tests and kits should be validated and be shown to be fit for purpose prior to use.

**Note:** Some commercial identification systems may misidentify *Brucella* species as *P. phenylpyruvicus*.

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

This has been shown to be a rapid and powerful tool because of its reproducibility, speed and sensitivity of analysis. The advantage of MALDI-TOF as compared with other identification methods is that the results of the analysis are available within a few hours rather than several days. The speed and the simplicity of sample preparation and result acquisition associated with minimal consumable costs make this method well suited for routine and high throughput use<sup>52</sup>.

The use of this technique for the distinguishing of *M. catarrhalis* subpopulations has helped to establish their role in colonization and disease manifestations, which is yet unknown. MALDI-TOF of intact *M. catarrhalis* has also provided a rapid and robust tool for *M. catarrhalis* strain typing that could be applied in epidemiological studies. The one factor limiting the use of MALDI-TOF MS remains the lack of a robust *M. catarrhalis* database which has hampered at the current time the determination of the corresponding protein or proteins<sup>53</sup>.

### 3.4.4 Nucleic Acid Amplification Tests (NAATs)

PCR is considered to be a good method as it is simple, sensitive and specific. The basis for PCR diagnostic applications in microbiology is the detection of infectious agents and the discrimination of non-pathogenic from pathogenic strains by virtue of specific genes.

This has been used for the rapid detection and quantification of *M. catarrhalis* in nasopharyngeal secretions without the need for bacterial culture by targeting the *copB* outer membrane protein gene; this may serve as a tool to study changes in the amounts of *M. catarrhalis* during lower respiratory tract infections<sup>54</sup>. This was specific only for *M. catarrhalis* and not for other *Moraxella* species.

PCR has also been reliably used for the detection of mixed bacterial infections, eg, with *M. catarrhalis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* in a single amplification assay. This approach has even been successful for culture negative effusion<sup>55</sup>. This has helped facilitate rapid diagnosis and prompt the initiation of the appropriate chemotherapy as well as used for epidemiological studies.

## 3.5 Further Identification

### Rapid Molecular Methods

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



A variety of rapid typing methods have been developed for isolates from clinical samples; these include molecular techniques such as Pulsed Field Gel Electrophoresis (PFGE), and 16S rRNA gene sequencing. All of these approaches enable subtyping of unrelated strains, but do so with different accuracy, discriminatory power, and reproducibility.

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

### **16S rRNA gene sequencing**

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

The availability of gene sequencing has revolutionized the taxonomy of the genus *Moraxella* and has been used to elucidate the relationships between species as well as decipher the placements of more distantly related *Moraxella* species and other members of the family belonging to the *Moraxellaceae*<sup>56</sup>.

This has also been used to identify new species; *Psychrobacter sanguinis*, as well as to update the description of already existing species; *Psychrobacter faecalis* and *Psychrobacter pulmonis* and also to re-classify organisms eg the transfer of *Kingella indologenes* to the Genus *Suttonella*<sup>17,28,32</sup>.

### **Pulsed Field Gel Electrophoresis (PFGE)**

PFGE detects genetic variation between strains using rare-cutting restriction endonucleases, followed by separation of the resulting large genomic fragments on an agarose gel. PFGE is known to be highly discriminatory and a frequently used technique for outbreak investigations and has gained broad application in characterizing epidemiologically related isolates. However, the stability of PFGE may be insufficient for reliable application in long-term epidemiological studies. However, due to its time-consuming nature (30hr or longer to perform) and its requirement for special equipment, PFGE is not used widely outside the reference laboratories<sup>57,58</sup>.

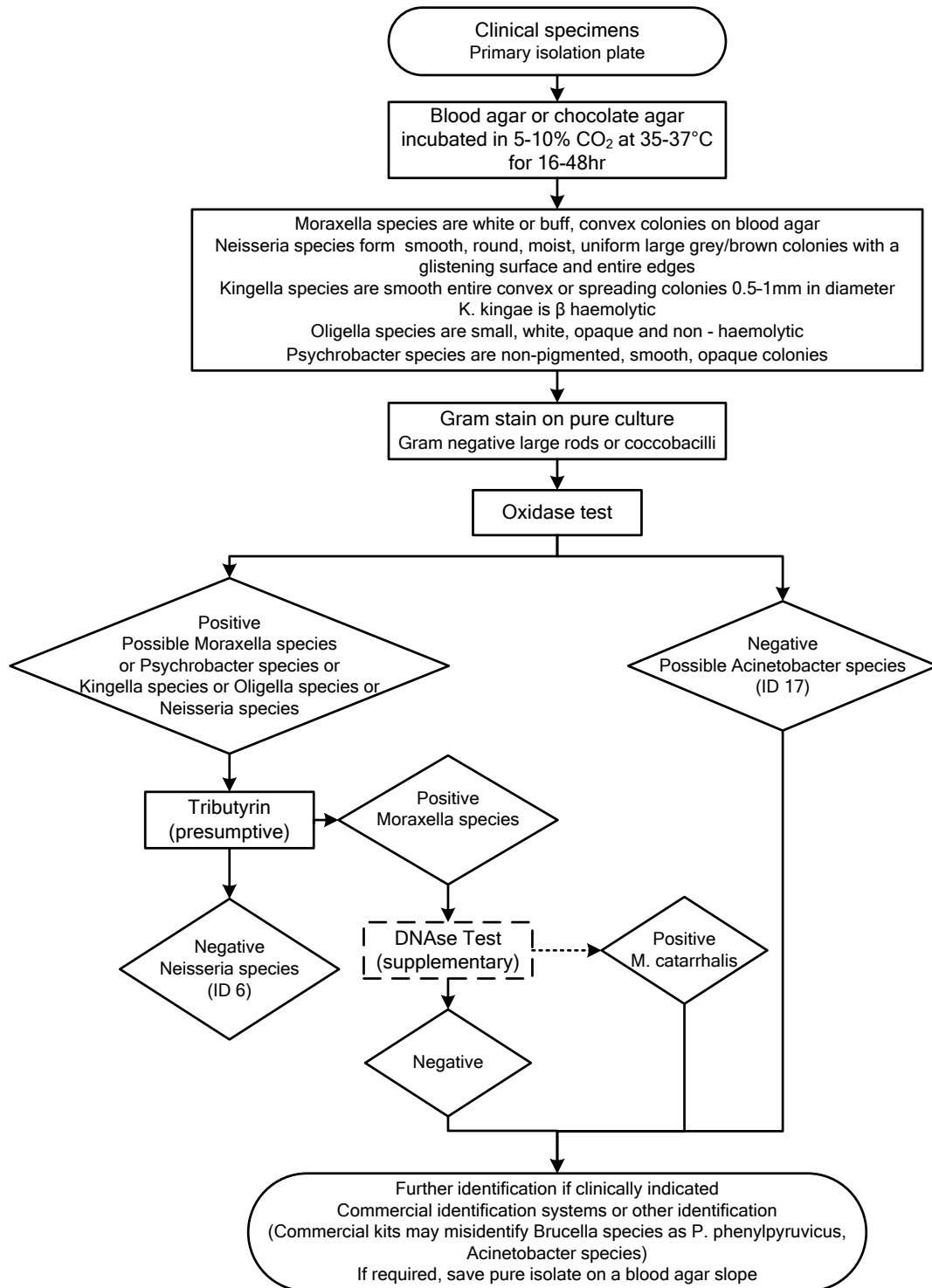
PFGE performed with *NotI* has been used to characterise strains of *M. catarrhalis* and an improved pulsed-field gel electrophoresis (PFGE) methodology determined *SpeI* as the best choice for typing *M. catarrhalis*, with a good restriction of clinical samples and a good clustering correlation with *NotI*<sup>59,60</sup>.

This has been helpful for understanding the spread of disease in both hospitals and communities.

## **3.6 Storage and Referral**

If required, save pure isolate on blood agar slopes for referral to the Reference Laboratory.

## 4 Identification of *Moraxella* species and Morphologically Similar Organisms



This flowchart is for guidance only.

## 5 Reporting

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### 5.1 Presumptive Identification

Demonstration of appropriate growth characteristics, colonial appearance, Gram stain of the culture, oxidase and tributyrin test result.

### 5.2 Confirmation of Identification

Following commercial identification kit or other biochemical test results.

### 5.3 Medical Microbiologist

The medical microbiologist should be informed of presumptive or confirmed *Moraxella* species and morphologically similar organisms when the isolate is from a normally sterile site or in cases of invasive disease.

Follow local protocols for reporting to clinician.

### 5.4 CCDC

Refer to local Memorandum of Understanding.

### 5.5 Public Health England<sup>61</sup>

Refer to current guidelines on CIDSC and COSURV reporting.

### 5.6 Infection Prevention and Control Team

N/A

## 6 Referrals

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### 6.1 Reference Laboratory

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

Laboratory of Healthcare Associated Infection  
Antimicrobial Monitoring and Health Care Associated Infections Reference Unit  
Microbiology Services  
Public Health England  
61 Colindale Avenue  
London  
NW9 5EQ

<https://www.gov.uk/amrhai-reference-unit-reference-and-diagnostic-services>

Contact PHE's main switchboard: Tel. +44 (0) 20 8200 4400

## 7 Notification to PHE<sup>61,62</sup> or Equivalent in the Devolved Administrations<sup>63-66</sup>

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The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are

listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

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

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

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

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

Other arrangements exist in [Scotland](#)<sup>63,64</sup>, [Wales](#)<sup>65</sup> and [Northern Ireland](#)<sup>66</sup>.

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## Identification of *Moraxella* species and Morphologically Similar Organisms

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