

NATIONAL STANDARD METHOD

IDENTIFICATION OF *MORAXELLA* SPECIES AND MORPHOLOGICALLY SIMILAR ORGANISMS

BSOP ID 11

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

Association of Medical Microbiologists
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IDENTIFICATION OF *MORAXELLA* SPECIES AND MORPHOLOGICALLY SIMILAR ORGANISMS

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AMENDMENT PROCEDURE

Controlled document reference	BSOP ID 11
Controlled document title	Identification of <i>Moraxella</i> species and morphologically similar organisms

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.

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/ 14/08/07	1.1	2	All 9 10 12	All Flow chart 6 Referrals References	Taxonomy updated Title changed and flowchart converted to Visio format. Contents of flow chart updated. Links to reference laboratory user manuals inserted. References reviewed and updated

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IDENTIFICATION OF *MORAXELLA* SPECIES AND MORPHOLOGICALLY SIMILAR ORGANISMS

SCOPE OF DOCUMENT

This National Standard Method (NSM) describes the identification of *Moraxella* species and those species which are morphologically similar. To differentiate *Moraxella* species from *Neisseria* species see BSOP ID 6 – Identification of *Neisseria* species <http://www.hpa-standardmethods.org.uk/documents/bsopid/pdf/bsopid6.pdf>. *Acinetobacter* species may also be misidentified as *Moraxella* species and their identification is described in BSOP ID 17 - Identification of glucose non-fermenting rods <http://www.hpa-standardmethods.org.uk/documents/bsopid/pdf/bsopid17.pdf>.

INTRODUCTION

Taxonomy

The genera *Moraxella* (including the former *Branhamella*), *Acinetobacter* and *Psychrobacter* currently belong to the family Moraxellaceae; the classification is currently under review. The genus *Oligella* includes the previous *Moraxella urethralis* and CDC group M-4 now both classified as *Oligella ureolytica* and these organisms are covered in BSOP ID 17 - Identification of glucose non-fermenting rods <http://www.hpa-standardmethods.org.uk/documents/bsopid/pdf/bsopid17.pdf>. *Kingella kingae* (formerly referred to as *Moraxella kingii* or *Moraxella* new species I) has been placed in the genus *Kingella*. *Kingella denitrificans*, previously designated CDC group TM-1, has also been placed in this genus and is described in BSOP ID 12 - Identification of *Haemophilus* species and the HACEK group of organisms <http://www.hpa-standardmethods.org.uk/documents/bsopid/pdf/bsopid12.pdf>.

Psychrobacter phenylpyruvicus (formally *Moraxella phenylpyruvica*) is phenotypically similar to *Moraxella lincolnii* and *M. osloensis*. *P. phenylpyruvicus* is often urease-positive (see BSOP TP 36 – Urease test) <http://www.hpa-standardmethods.org.uk/documents/bsopTP/pdf/bsoptp36.pdf>. *Brucella* species can be misidentified as *P. phenylpyruvicus* in some commercial identification kits. *Psychrobacter* species are described in BSOP 17 - Identification of glucose non-fermenting rods <http://www.hpa-standardmethods.org.uk/documents/bsop/pdf/bsop17.pdf>.

Characteristics

Genus *Moraxella*

Moraxella species are Gram-negative and 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. The optimum growth temperature is 33°C – 35°C. *Moraxella* species are usually catalase-positive, oxidase positive and do not produce acid from carbohydrates. Colonies of *Moraxella lacunata* and *Moraxella nonliquefaciens* are small on blood agar. Some strains of *M. lacunata* are haemolytic. *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 negative²⁻⁴. However, as the tributyrin test is positive for *Moraxella* species other than *M. catarrhalis*, it cannot be used alone to differentiate among the Moraxellae²⁻⁴. Ninety percent of *M. catarrhalis* are β-lactamase positive.

The genus is divided into *Moraxella* subgenus *Moraxella* which includes all the rod shaped species, and *Moraxella* subgenus *Branhamella* which contains the cocci.

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Moraxella* subgenus *Moraxella

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 are *Moraxella atlantae*, *M. lacunata*, *M. nonliquefaciens* and *M. osloensis*.

Moraxella* subgenus *Branhamella

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

Principles of identification

Colonies isolated on chocolate or blood agar plates are identified by colonial morphology, Gram's 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

N/A

1 SAFETY CONSIDERATIONS⁵⁻¹⁵

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

***Moraxella* species and morphologically similar organisms reported to have caused human infection^{16,17}**

M. atlantae

M. catarrhalis

M. lacunata

M. nonliquefaciens

M. osloensis

K. denitrificans

K. kingae

O. urethralis

P. immobilis

P. phenylpyruvicus

3 IDENTIFICATION

3.1 MICROSCOPIC APPEARANCE

Gram stain (BSOP TP 39 – Staining Procedures) <http://www.hpa-standardmethods.org.uk/documents/bsopTP/pdf/bsoptp39.pdf>

Gram-negative with a tendency to resist decolourisation.

Moraxella* subgenus *Moraxella

Rods, often coccobacilli. Usually occur in pairs or short chains with one plane of division.

Moraxella* subgenus *Branhamella

Cocci occur singly or in pairs with adjacent sides flattened, sometimes 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 phenylpyruvicus

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 16 – 48 h incubation in 5 - 10% CO₂ at 35°C - 37°C

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3.3 COLONIAL APPEARANCE

Moraxella* subgenus *Moraxella

Smooth, flat, uniform, buff colonies 1 – 2 mm 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.

Moraxella* subgenus *Branhamella

Smooth, round, uniform, grey/brown colonies 1 mm in diameter.

***Kingella* species**

Two types of colonies occur on blood agar, a smooth entire convex type and a spreading colony. Colonies are small, 0.5 – 1 mm in diameter after 48 h. *K. kingae* produce distinct zones of beta-haemolysis.

***Oligella* species**

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

Psychrobacter phenylpyruvicus

Require incubation at 20°C – 25°C. Colonies of *P. phenylpyruvicus* are small on blood agar. Growth is enhanced by bile salts to form non-pigmented, smooth, opaque colonies.

3.4 TEST PROCEDURES

Oxidase test (BSOP TP 26 – Oxidase test)

<http://www.hpa-standardmethods.org.uk/documents/bsopTP/pdf/bsoptp26.pdf>

Positive

Tributylin test

Positive

DNase test (BSOP TP 12 – Deoxyribonuclease test)

<http://www.hpa-standardmethods.org.uk/documents/bsopTP/pdf/bsoptp12.pdf>

Positive for *M. catarrhalis*

Commercial identification kit

Additional biochemical/other tests. Commercial kits may misidentify *Brucella* species as *P. phenylpyruvicus*.

3.5 FURTHER IDENTIFICATION

N/A

3.6 STORAGE AND REFERRAL

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

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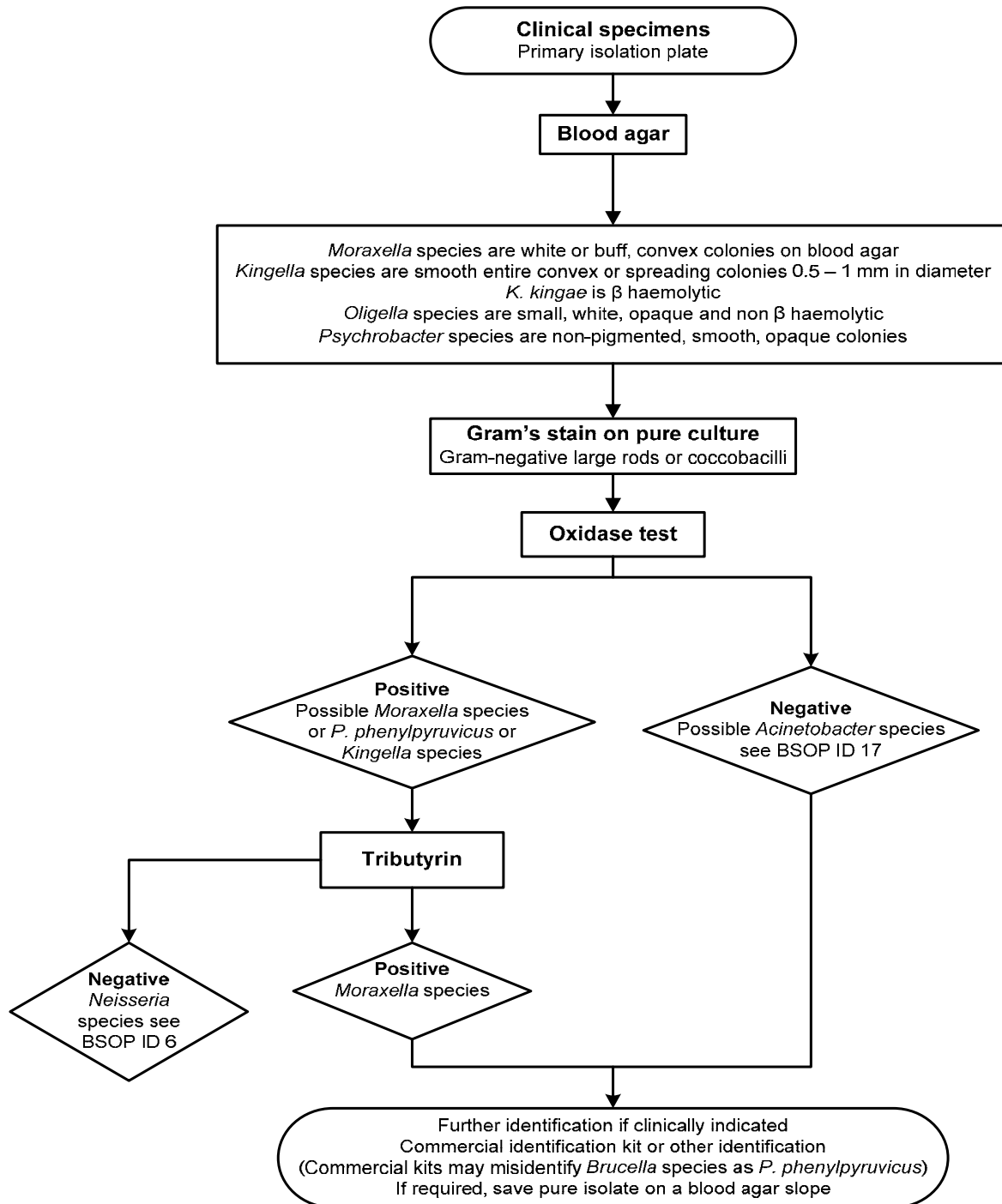
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4 IDENTIFICATION OF MORAXELLA AND MORPHOLOGICALLY SIMILAR SPECIES – FLOW CHART



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5 RESULTS AND REPORTING

5.1 PRESUMPTIVE IDENTIFICATION

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

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 CFI¹⁸

Refer to current guidelines on CDSC and COSURV reporting.

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/reference_tests_index.htm

Laboratory of Healthcare Associated Infection
Centre for Infections
Health Protection Agency
61 Colindale Avenue
London
NW9 5HT

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

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7 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the National Standard Methods Working Group for Clinical Bacteriology (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.

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For further information please contact us at:

Standards Unit
Evaluations and Standards Laboratory
Centre for Infections
Health Protection Agency
Colindale
London
NW9 5EQ
E-mail: standards@hpa.org.uk

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