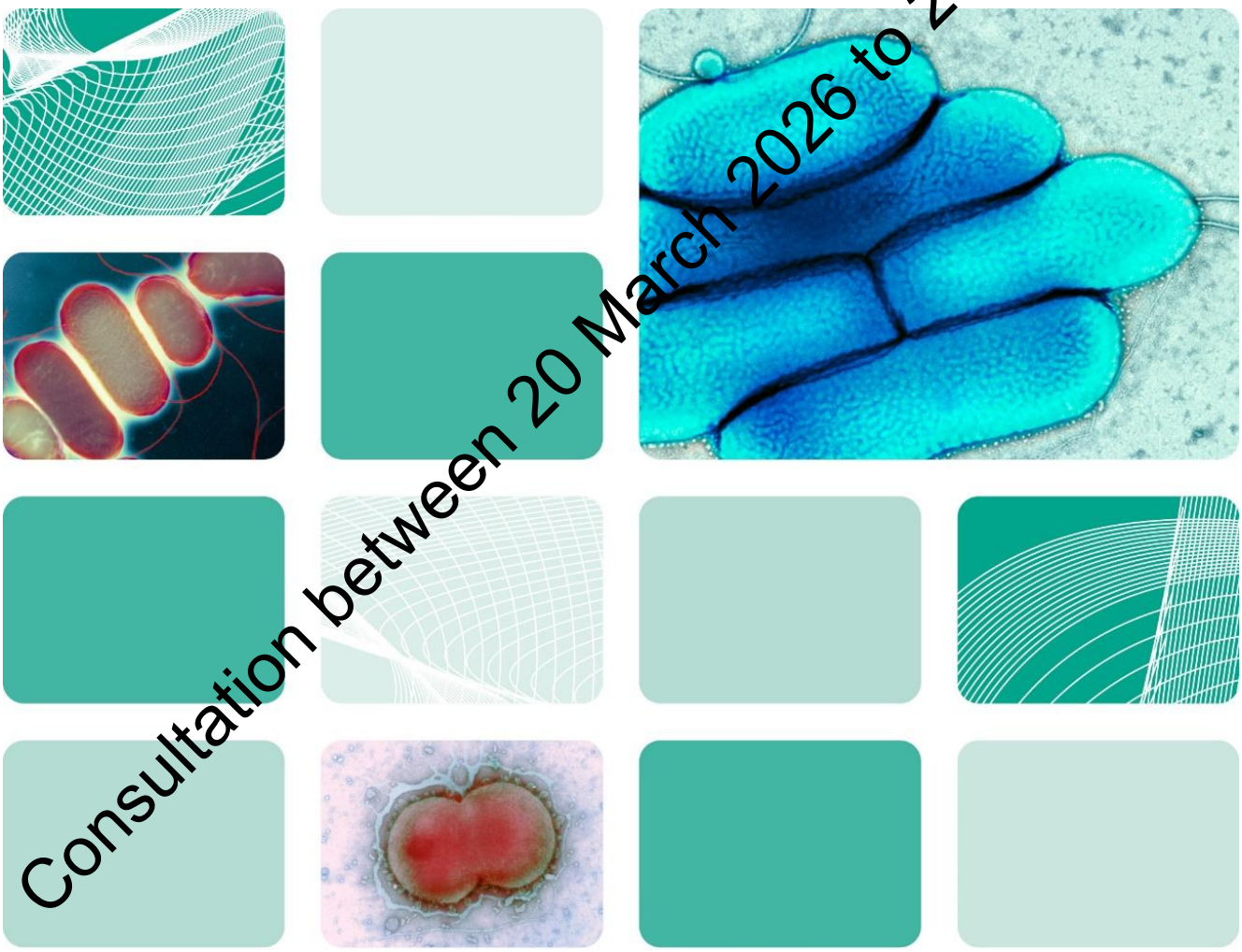
UK Health Security Agency

UK Standards for Microbiology Investigations

Identification of *Moraxella* species and morphologically similar organisms



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Contents

Amendment table

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*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 *Moraxella* species and other morphologically similar organisms such as *Oligella* species and *Psychrobacter* species, using methods such as microscopy, matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) and commercial identification systems or phenomic approaches as required. Molecular methods are mentioned as an alternative method for identification and confirmation.

Please note, the use of some biochemical tests may not be required except in cases where confirmation by an alternative technique is needed or where automated methods are not available.

This document mentions the differentiation of *Moraxella* species from *Neisseria, Kingella* and *Acinetobacter* species. The identification of these genera are covered in UK SMI ID 6 – Identification of *Neisseria* species, UK SMI ID 12 - Identification of *Haemophilus* species and the HACEK group of organisms and UK SMI ID 17 - Identification of *Pseudomonas* species and morphologically similar organisms

For further information on specific organisms and their associated clinical syndromes, please refer to the relevant UK SMI in the Syndromic and Bacteriology categories.

This UK SMI should be used in conjunction with other UK SMIs.

4 Introduction

4.1 Target organisms of clinical significance

Moraxella species are generally considered commensals of human mucous membranes, including the respiratory and genital tract, however, some species can cause disease. The most clinically relevant species is *Moraxella catarrhalis,* which is associated with acute otitis media in children and lower respiratory infections associated with chronic obstructive pulmonary disease (COPD), pneumonia and sinusitis in adults (1-3). Colonisation by *M. catarrhalis,* is common in children, with the

incidence decreasing with age. Colonisation is also more common in patients with COPD and is linked to COPD exacerbation (2).

Moraxella osloensis is commonly isolated in blood cultures. Infections caused by *M. osloensis* include endocarditis, meningitis, osteomyelitis, septic arthritis, vaginitis, and bacteraemia (4). Isolated *M. osloensis* bacteraemia is an uncommon clinical finding in immune competent patients. *M. osloensis* and *Moraxella nonliquifaciens* ocular bacterial pathogens isolated in cases of keratitis, conjunctivitis, and endophthalmitis (4,5). *M. nonliquefaciens* can also be isolated from the human respiratory tract (6).

Moraxella lacunata is the main species that represents the genus, although it is not as frequently isolated as *M. catarrhalis* (7). *M. lacunata* has been implicated in conjunctivitis, endocarditis and keratitis (6,8). Other *Moraxella* species, including *Moraxella atlantae* and *Moraxella lincolnii*, have been isolated from humans, are of less clinical relevance (6).

Of the other morphologically similar organisms, the genus *Oligella* only contains 2 species: *Oligella urethralis* and *Oligella ureolytica* (7). They are mainly isolated from the genitourinary tract of humans and are unlikely to be pathogenic, although they have been implicated in invasive disease in patients that are immunosuppressed or have underlying health conditions (9).

Psychrobacter species are found in a variety of habitats, ranging from marine environments to human microbiota, making them interesting organisms for the medical profession as well as microbiological and environmental research (10). They are considered part of the human microbiota. Human infection by *Psychrobacter* species is rare, with only 6 species known to have been isolated in humans at the time of review. These are *P. arenosus*, *P. immobilis, P. faecalis, P. phenylpyruvicus, P. pulmonis* and *P. sanguinis.*

4.2 Taxonomy and characteristics

Moraxella species are Gram negative rods or coccobacilli belonging to the *Moraxellaceae* family (7). *Moraxella* species are non-motile and aerobic, but some strains may grow weakly under anaerobic conditions. Most species, except *M. osloensis*, are nutritionally fastidious. Growth on standard media may be sparse or fail completely and generally demonstrate a lack of colony pigmentation Some species are stimulated significantly by fatty acids (bile salts, Tween 80). The optimum growth temperature is 33 - 35°C. Pleomorphism is enhanced by lack of oxygen and by incubation at temperatures above the optimum (6). They are oxidase and catalase positive.

Oligella species are small rods, often occurring in pairs. The cells lack the plumpness of *Moraxella* species. They are aerobic, non-capsulated, non-spore-forming and mostly non-motile, but some strains of *O. ureolytica* are peritrichously flagellated (11).

Psychrobacter cells are non-motile, Gram negative coccobacilli. Unlike *Moraxella* species, many *Psychrobacter* species can form acid aerobically from glucose and

several other sugars. They are psychrotrophic and therefore can grow at temperatures as low as 5°C, although optimal growth occurs closer to 20°C. They are generally unable to grow at 35 - 37°C, although some strains have an optimal growth at these temperatures.

5 Safety considerations

The section covers specific safety considerations (12-33) related to this UK SMI and should be read in conjunction with the general safety considerations.

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

M. catarrhalis is a Hazard group 2 organism, the processing of diagnostic samples should be carried out at Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet.

Consider *Neisseria meningitidis* from respiratory samples. All work on suspected *N. meningitidis* which is likely to generate aerosols must be performed 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.

6 Identification

In routine laboratory workflows, MALDI-TOF MS is overwhelmingly the primary method used for species-level identification. When MALDI-TOF MS results are inconclusive, confirmation is required, or automated systems are unavailable, alternative characterisation may be achieved using phenotypic or biochemical tests performed from non-selective media. Real-time PCR and other NAAT platforms, may also be used when clinically appropriate.

6.1 Microscopical appearance

Gram stain

(TP 39 - Staining Procedures)

All species are Gram negative with a tendency to resist decolourisation.

- Some *Moraxella* species, such as *M. catarrhalis,* are coccobacilli usually occurring in pairs or short chains with one plane of division. They may also appear as cocci occurring singly or in pairs with adjacent sides flattened,

forming tetrads. Other *Moraxella* species may be rod-shaped, such as *M. nonliquefaciens.*

- *Oligella* species are small rods or coccobacilli, often occurring in pairs. Cells lack the typical plumpness of *Moraxella* species.
- *Psychrobacter* species are rods, often coccobacilli that usually occur in planes with one plane of division. Microscopy can differentiate *Brucella* species (very small coccobacilli) from *P. phenylpyruvicus*.

6.2 Primary isolation media

Moraxella species, *Oligella* species and *Psychrobacter* species can grow on a range of media including CLED and chocolate agar, but the primary isolation media is blood agar. CO_2 is required for optimal growth. *Psychrobacter* species require incubation at 20°C - 25°C.

6.3 Colonial appearance

Moraxella species

Colonies vary with species. *M. catarrhalis* colonies are generally uniform, smooth, convex, buff and 1 - 2mm in diameter. *M catarrhalis* colonies are positive for the hockey puck test, where colonies are cohesive enough that they can be pushed across the agar surface without collapsing. This is not seen in *Neisseria, Oligella or Psychrobacter* species.

For colonial appearance of non-catarrhalis species, please see Table 1.

Note: On chocolate agar, colonies of *M. catarrhalis* are pinkish brown, and may resemble *N. gonorrhoeae*.

Oligella species

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

Psychrobacter species

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.

Table 1: Microscopic and colonial appearance of *Moraxella* species and other morphologically similar species (6,11,34)

Species	Microscopic and colonial characteristics
M. catarrhalis	Cocci with division in 2 planes. May be fimbriated. Colonies are buff, opaque and non-haemolytic. Characterised by non-adherent colonies that slide across the agar when pushed.
M. atlantae	Variably sized diplococcobacillary to red-shaped cells that are often fimbriated. Colonies are small, non-haemolytic and slightly opaque and show spreading/pitting of agar.
M. lacunata,	Thick/plump rods, 0.8 - 1.2µm in diameter, occurring in diploid pairs and chains. Tendency to lose their Gram negative staining characteristics when left out for days. Small, semi opaque colonies that may pit agar.
M. nonliquefaciens	Plump rods with obtuse nearly square ends, often very short diplobacilli. Small, low convex colonies that occasionally spread and pit the agar. Non-pigmented and non-haemolytic.
M. osloensis	Shaped like *M. nonliquefaciens,* however some may show a more fusiform or lanceolate shape. Colonies appear similar to *M. lincolnii* but pitting of the agar is rare.
M. lincolnii	Plump rods, often occurring in pairs and may form short chains. Colonies are whitish, smooth, convex and circular. Some strains may have a flattened edge.
Oligella species	Small rods, generally not exceeding 1 µm and often occur in pairs. Colonies develop slowly and more overtly white than *Moraxella*. Non-haemolytic.
P. immobilis	Plump coccobacilli frequently showing diploforms.
P. phenylpyruvicus,	Coccoid. Circular, slightly convex, cream-coloured colonies. Psychrotolerant and halotolerant
P. faecalis,	Straight rods, occurring singly. On nutrient agar, colonies are circular, opaque, slightly raised and beige.
P. pulmonis,	Coccus-shaped. Non-pigmented, smooth colonies on blood agar.
P. sanguinis,	Non-haemolytic, non-pigmented coccobacilli. Colonies are moist, circular and smooth.
P. aerodus	Ovoid cells. On blood agar colonies are monomorphic, small and grey. On TSA colonies are opaque, circular, convex and cream coloured. Psychrotolerant

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

MALDI-TOF MS is used to provide an accurate and rapid primary identification of *Moraxella, Oligella* and *Psychrobacter* species (35). This method should be appropriately validated, and all databases updated. Manufacturer's instructions should be followed. The use of an extraction step for more reliable identification should be considered.

Laboratories should follow local policies on whether MALDI-TOF MS is used primarily for identification, but any low identification scores should be retested with other biochemical tests

6.5 Further identification

6.5.1 Biochemical tests

Oxidase test

(TP 26 – Oxidase Test)

Moraxella species are oxidase positive and may be misidentified as other oxidase positive isolates such as *Pseudomonas* species, *Neisseria* species and *Kingella* species.

Tributyrin test

2-4hr Presumptive test

Moraxella species are tributyrin positive and can be used to differentiate *M. catarrhalis* from *Neisseria* species which are negative (36).

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.

6.5.2 Commercial identification Systems

Other identification systems including commercial kits may be used as a supplementary or confirmatory 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* (37).

6.5.3 Molecular Methods

If identification using the previously described methodologies is not possible, 16S rRNA gene sequencing can be used for identification where required.

16S rRNA may also be beneficial for detection of *Moraxella* and other morphologically similar organisms directly from sterile clinical samples.

NAATs may be used for the rapid detection and quantification of *M. catarrhalis* in nasopharyngeal secretions or other validated sample types without the need for bacterial culture and when investigating mixed bacterial infection, e.g. with *M. catarrhalis, Haemophilus influenzae,* and *Streptococcus pneumoniae* in a single amplification assay.

6.6 Storage and referral

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

7 Reporting

7.1 Designated Infection Specialist

The infection specialist 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.

7.2 Health Protection Team (HPT)

Refer to local agreements in devolved administrations.

7.3 UK Health Security Agency

Refer to current guidelines on Second Generation Surveillance System (SGSS) reporting (28).

7.4 Infection prevention and control team

N/A

8 Referral to reference laboratories

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

Contact appropriate reference 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.

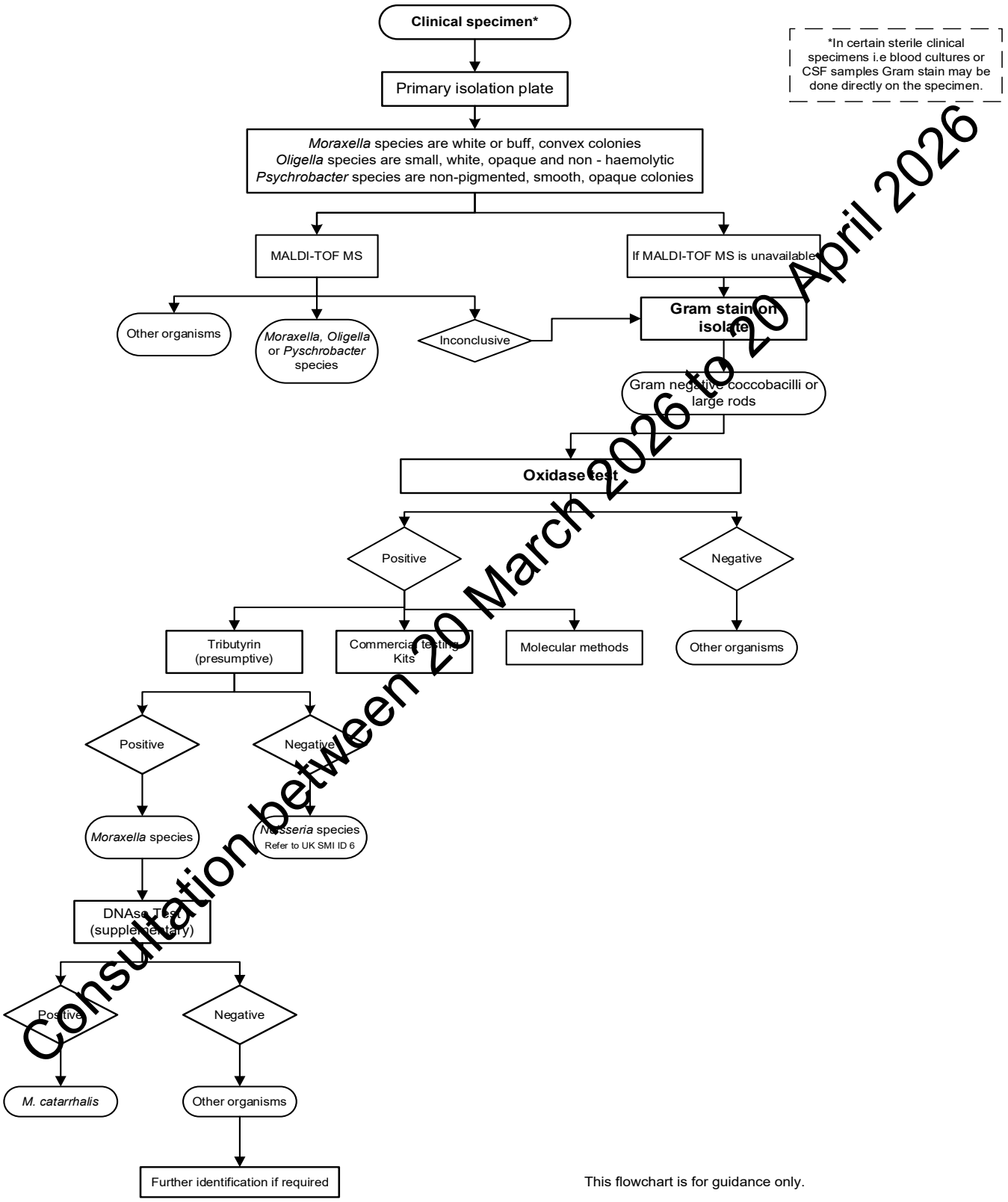
9 Public health responsibilities of diagnostic laboratories

Diagnostic laboratories have public health responsibility as part of their duties. Amongst these are additional local testing or referral to further characterise the organism as required, primarily for public health purposes e.g., routine cryptosporidium detection; serotyping or microbial subtyping; and a duty to refer appropriate specimens and isolates of public health importance to a reference laboratory.

Diagnostic laboratory outputs inform public health intervention, and surveillance data is required to develop policy and guidance forming an essential component of healthcare. It is recognised that additional testing and referral of samples may entail some costs that has to be borne by the laboratory but in certain jurisdictions these costs are covered centrally.

Diagnostic laboratories should be mindful of the impact of laboratory investigations on public health and consider requests from the reference laboratories for specimen referral or enhanced information.

Algorithm: Identification of *Moraxella* species and Morphologically Similar Organisms



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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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