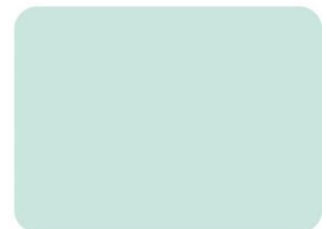
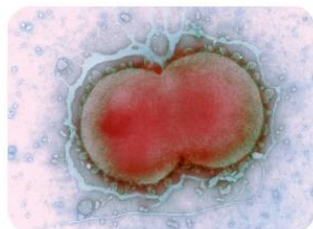
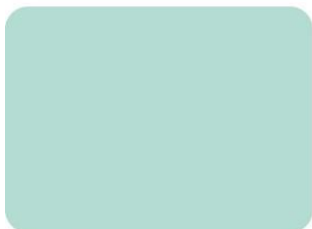
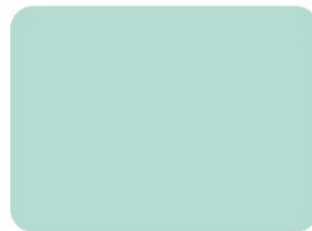
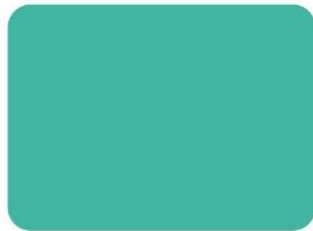
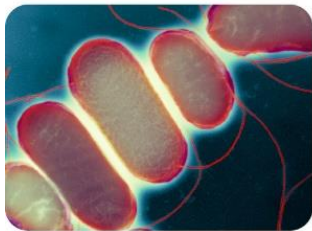
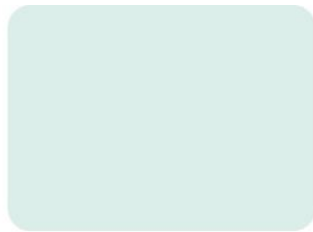
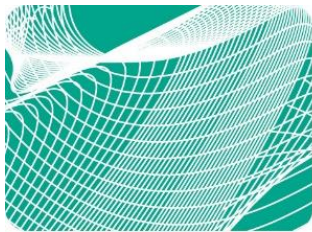




UK Health
Security
Agency

UK Standards for Microbiology Investigations

Identification of *Neisseria* species



Acknowledgments

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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.

UK SMIs are produced in association with:



Displayed logos correct as of December 2024

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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	9/11.07.25
Issue number discarded	4
Insert issue number	4.1
Section(s) involved	Amendment
Whole document	<p>This is an administrative point change.</p> <p>The content of this UK SMI document has not changed.</p> <p>The last scientific and clinical review was conducted on 26/02/2024.</p> <p>Hyperlinks throughout document updated to Royal College of Pathologists website.</p> <p>Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms</p> <p>Partner organisation logos updated.</p> <p>Broken links to devolved administrations replaced.</p> <p>References to NICE accreditation removed.</p> <p>Scope and Purpose replaced with General and Scientific information to align with current UK SMI template.</p>

Amendment number/date	8/26.02.24
Issue number discarded	3
Insert issue number	4
Anticipated next review date*	26.02.27
Section(s) involved	Amendment

<p>Whole document</p>	<p>Hyperlinks updated to direct reader to UK SMIs on the RCPATH website rather than GOV.UK.</p> <p>Subheadings have been revised and modified as required.</p> <p>All sections have been updated with current and relevant information and supported with recent literature where available.</p> <p>Some sections have been restructured as appropriate to align with current laboratory practices.</p>
<p>Scope of document</p>	<p>The scope has been updated to list the identification methods covered in the document.</p> <p>Links to other relevant UK SMIs that can be read in conjunction with this document have been added – UK SMI TP 40.</p> <p>Topics that are outside the scope of this document have been mentioned and links to relevant UK SMIs were provided if available – UK SMI B 51.</p> <p>The reader is also made aware of the reclassification and updated nomenclature of some <i>Neisseria</i> species mentioned in the document.</p>
<p>Introduction</p>	<p>The taxonomy of <i>Neisseria</i> species has been updated.</p> <p>The information under the characteristics section on <i>Neisseria</i> species has been summarised in Table 1.</p>
<p>Technical information and limitations</p>	<p>The information under this section has either been moved to Section 8: Identification or removed if not relevant anymore.</p>
<p>Safety considerations</p>	<p>The information on the vaccines available for meningococcal disease and the transmission routes in a laboratory setting have been updated.</p>
<p>Identification</p>	<p>Table 1 added to summarise the microscopic and colonial appearance of the target <i>Neisseria</i> species.</p>
<p>Reporting</p>	<p>The information has been updated as appropriate.</p>
<p>Referral to reference or specialist testing laboratories</p>	<p>Hyperlinks were updated as appropriate.</p>

Algorithm	The structure and content of the algorithm has been updated to align with the current state of laboratory practices and knowledge.
References	References reviewed and updated.

*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 SMIs) document describes the identification of *Neisseria* species using culture, microscopy, oxidase test and matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for identification. 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 conventional and molecular methods for alternative identification and confirmation.

This document describes the differentiation of pathogenic *Neisseria* from non-pathogenic *Neisseria* species and the related genera of *Moraxella* and *Kingella*. The identification of these genera are covered in [UK SMI ID 11: Identification of Moraxella species and morphologically similar organisms](#) and [UK SMI ID 12: Identification of Haemophilus species and the HACEK group of organisms](#).

This document does not focus on the molecular detection of *Neisseria gonorrhoeae* and *Neisseria meningitidis* or antimicrobial susceptibility testing of *Neisseria* species. The screening of *Neisseria meningitidis* is covered in [UK SMI B 51: Screening for Neisseria meningitidis](#).

Please note that some of the *Neisseria* species have been reclassified, and the updated nomenclature of these species have been included in this document for reference.

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

4 Introduction

4.1 Taxonomy and characteristics

The genus *Neisseria* comprises Gram-negative bacteria belonging to the family Neisseriaceae, order Neisseriales within the phylum β -Proteobacteria (1-4). There are currently more than 30 *Neisseria* species and 3 subspecies that may be isolated from humans and animals (1,2). The following species: *Neisseria ovis*, *Neisseria cuniculi*

and *Neisseria caviae* have been reclassified to *Moraxella ovis*, *Moraxella cuniculi* and *Moraxella caviae*, respectively (1,5,6).

The clinically important species are *Neisseria gonorrhoeae* and *Neisseria meningitidis*. These species are closely related but cause entirely different diseases with distinct clinical pathologies (7). *N. gonorrhoeae* is an obligate pathogen that causes the sexually transmitted infection (STI) gonorrhoea. *N. meningitidis* is an opportunistic pathogen that colonises the nasopharyngeal mucosa and has the potential to cause meningococcal disease which includes meningitis and septicemia (7).

The other *Neisseria* species such as *Neisseria lactamica* and *Neisseria cinerea* are generally considered commensals, but have been implicated as causes of infection in patients who are immunocompromised (8). More recent *Neisseria* species implicated in human disease include *Neisseria brasiliensis*, *Neisseria dumasiana*, *Neisseria oralis*, *Neisseria shayeganii*, *Neisseria wadsworthii* and *Neisseria skkuensis* (1,9-13).

Neisseria species are Gram-negative cocci, 0.6 - 1.0 µm in diameter, occurring singly but more often in pairs with adjacent sides flattened; except *Neisseria elongata*, *Neisseria weaver*, *Neisseria bacilliformis* and *Neisseria shayeganii*. These species consist of rods, 0.5 µm wide, non-motile, often arranged as diplococci or in short chains (7,12,14-16). Some species produce a greenish-yellow carotenoid pigment and some may be nutritionally fastidious and haemolytic (7). Some species are also saccharolytic. The optimum growth temperature is 35 - 37°C. *Neisseria* species are oxidase positive and catalase positive (except *Neisseria elongata*) (7).

5 Technical information and limitations

The advancement in molecular typing revealed that *Neisseria* species are larger and more diverse than previously thought (1,16). This led to the discovery of many novel species and the reclassification and nomenclature changes of others (1,5).

The changes made in the taxonomy of the *Neisseria* genus need to be reflected in the databases of the identification tools used in laboratories. This is particularly important for species that are closely related to *N. gonorrhoeae* and *N. meningitidis* because misidentification can have a serious health, legal and social consequences (17-19).

Note: The social consequences to the patient and the organisation of an incorrect diagnosis of gonorrhoea disease as a result of misidentification should not be underestimated.

6 Safety considerations

This section covers specific safety considerations (20-40) related to this UK SMI and should be read in conjunction with the general [safety considerations](#).

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

Due to the severity of the disease and the risks associated with generating aerosols, any manipulation of suspected isolates of *N. meningitidis* should always be undertaken in a microbiological safety cabinet until *N. meningitidis* has been ruled out (as must any laboratory procedure giving rise to infectious aerosols) (36).

N. meningitidis causes severe and sometimes fatal disease. Laboratory acquired infections have been reported (41,42). The organism infects primarily by the respiratory route. An effective vaccine is available for most meningococcal groups. Vaccination is required for laboratory staff routinely working with the organism.

N. gonorrhoeae is also a Hazard group 2 organism which is responsible for the STI gonorrhoea. It can also cause eye or throat infection - which poses a risk to laboratory workers through either direct inoculation, poor laboratory practice or inhalation of aerosols.

Refer to the 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.

7 Target organisms

Please refer to Section 8.2.1, Table 1 for *Neisseria* species that have been associated with human disease.

Other organisms which may be misidentified as *Neisseria* species are *Moraxella catarrhalis* and *Kingella denitrificans* (5,43).

8 Identification

In clinical laboratories, the identification of *Neisseria* species typically involves a combination of methods. Culture-based methods are primarily used for identification, with the integration of faster identification techniques such as MALDI-TOF MS, improving accuracy of identification. Conventional and molecular methods can provide an alternative means of identification and be used for confirmation where appropriate.

8.1 Culture methods

Culture methods provide presumptive identification of *Neisseria* species based on colony morphology (in some cases - Gram stain) and oxidase, followed by identification via MALDI-TOF MS.

8.1.1 Bacterial growth media

Some *Neisseria* species including *N. gonorrhoeae* and *N. meningitidis* are fastidious and require enriched media for growth. They grow best in aerobic conditions at temperatures of 35 – 37°C with 5 - 10% CO₂ (7,44). Colonies usually appear within 18 - 48 hours of incubation and vary in morphological appearance (7).

8.1.1.1 Primary agar

Whole blood or heated blood (chocolate) agar incubated for 18 - 48 hours in 5 - 10% CO₂ at 35 - 37°C (7,18). The media usually consist of Columbia agar base supplemented with 5% horse blood or chocolatised horse blood (7).

Note: *N. gonorrhoeae* grows poorly on whole blood agar, so a non-selective GC agar with lysed or chocolatised horse blood, and/or 1% Vitox or IsoVitaleX should be used.

8.1.1.2 Selective agar

GC selective agar incubated for 18 - 48 hours in 5 - 10% CO₂ at 35-37°C (7). This selective agar is primarily used for the selective isolation of *N. gonorrhoeae* but can also be used for the isolation of *N. meningitidis*.

Note: Non-pathogenic *Neisseria* species generally do not grow well on GC or Thayer-Martin selective agar. For the isolation of these species, modified selective agar including Modified Thayer-Martin (MTM) agar may be a more suitable option.

8.1.2 Colonial appearance

Neisseria species are usually pigmented and opaque. However, both *N. gonorrhoeae* and *N. meningitidis* form smooth, round, moist, uniform grey/brown colonies with a greenish colour on primary isolation medium (7).

8.2 Microscopic appearance

8.2.1 Gram stain

Please refer to [UK SMI TP 39: Staining procedures](#).

Neisseria species - Gram-negative cocci arranged in pairs with long axes parallel or Gram-negative rods that are arranged in chains or as diplococci (7). Refer to Table 1 for the colonial and microscopic appearance of *Neisseria* species.

Note: Gram stain is often omitted in primary diagnostic laboratories from the identification process if isolates are going to be identified using MALDI-TOF MS.

Table 1. Microscopic and colonial morphology of *Neisseria* species (1,5-7,12,13,44-60).

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

Species	Colonies	Additional comments
<i>Neisseria gonorrhoeae</i>	Diplococci with concave adjacent sides. Smooth, round, moist, uniform grey/brown with a greenish colour.	Non-haemolytic. No pigmentation. Poor growth on whole blood agar when the medium is very fresh, or the number of bacteria present in the sample is especially high. Autolysis and sticky colonies with prolonged growth.
<i>Neisseria meningitidis</i>	Diplococci. Similar to <i>N. gonorrhoeae</i>	Non-haemolytic on blood agar. No pigmentation. Autolysis with prolonged growth.
<i>Neisseria lactamica</i>	Diplococci. Colonies less moist and smaller than <i>N. gonorrhoeae</i> and <i>N. meningitidis</i> .	Haemolytic on horse blood agar*. Yellow pigmentation.
<i>Neisseria cinerea</i>	Diplococci/scattered clusters. Small, greyish white colonies with entire edges, and slightly granular structure.	Non-haemolytic. Yellow pigmentation*.
<i>Neisseria elongata</i>	Small slender rods that occur in chains. Greyish white shiny opaque colonies, low-hemispherical with an entire edge.	Non-haemolytic with some pitting of the agar. Yellow pigmentation*.
<i>Neisseria elongata</i> subsp. <i>elongata</i>	Flat colonies.	Non-haemolytic.
<i>Neisseria elongata</i> subsp. <i>glycolytica</i>	Similar to <i>N. elongata</i> colonies.	Haemolysis varies. Yellow pigmentation*. Relatively large grey, opaque, moderately raised with flat top and smooth with a soft homogenous consistency on blood agar.
<i>Neisseria elongata</i> subsp. <i>nitroreducens</i>	Similar to <i>N. elongata</i> colonies.	None.
<i>Neisseria sicca</i>	Cocci occurring in pairs and tetrads. Small round colonies, having a smooth surface and an entire edge.	Haemolytic*. Yellow pigmentation*. Colonies increase in size, and appear raised, rough, and black after 24 hrs. Very firm to the medium.

Identification of *Neisseria* species

Species	Colonies	Additional comments
<i>Neisseria mucosa</i>	Diplococci. Large, mucoid, and often adherent.	No haemolysis*. No pigmentation or greyish to buff yellow.
<i>Neisseria canis</i>	Diplococci/rarely in tetrads. Smooth, butyrous with a light-yellow tinge.	No haemolysis. No pigmentation.
<i>Neisseria flava</i>	Diplococci. Discrete, opaque, pale-yellow, slightly flatter than <i>N. meningitidis</i> colonies.	Yellow pigmentation.*
<i>Neisseria subflava</i>	Cocci occurring in pairs and tetrads. Smooth, transparent, or opaque, and often adherent.	No haemolysis. Yellow pigmentation. They tend to resist Gram decolourisation.
<i>Neisseria bacilliformis</i>	Small rods. Round, smooth, glistening, light grey colonies.	None.
<i>Neisseria weaveri</i>	Broad, plump, medium to large, straight rods of varying length in chains or longer rods. Smooth, flat, somewhat glistening with an entire border.	Haemolytic. Colonies are variable in size and increase after 24 hrs.
<i>Neisseria flavescens</i>	Cocci occurring in pairs or tetrads. Smooth and opaque.	Non-haemolytic. Golden/yellow pigmentation.
<i>Neisseria oralis</i>	Cocci occurring in chains. Small, circular, entire, raised, moist and yellow.	Weak haemolysis.
<i>Neisseria shayeganii</i>	Rod-shaped and long. Small, circular, entire, convex, moist, light-yellow grey.	Non-haemolytic.
<i>Neisseria wadsworthii</i>	Diplococci in chains. Small, circular, entire, convex, moist, light-yellow orange	Non-haemolytic.
<i>Neisseria zoodegmatis</i>	Cocci rods. Circular, convex, entire, opaque, shiny and smooth.	Haemolytic, with no pigmentation.
<i>Neisseria animaloris</i>	Cocci rods. Colonies are circular, convex, entire, opaque, shiny and smooth.	Haemolytic with no pigmentation.

Species	Colonies	Additional comments
<i>Neisseria dumasiana</i>	Coccioid to coccobacilli, may be present in pairs. Grey, moist, circular and convex.	Non-haemolytic. Grey pigmentation
<i>Neisseria brasiliensis</i>	Diplococci and brownish colonies.	None.
<i>Neisseria Skkuensis</i>	Small, round, and light grey.	None.
<i>Neisseria polysaccharea</i>	Cocci arranged in pairs or tetrads. Relatively small (2mm) yellowish colonies.	Non-haemolytic. Large amounts of polysaccharides produced.
<i>Neisseria caviae</i> *	Diplococci with adjacent sides flattened. Small (2mm), circular, convex with entire edge, and a smooth glistening surface. Butyrous becoming viscid.	Weakly haemolytic* with light caramel-light brown pigmentation.
<i>Neisseria cuniculi</i> *	Oval cocci, small and smooth	Haemolytic
<i>Neisseria ovis</i> *	Diplococci, grey, opaque and convex	Haemolytic.
* Reclassified at time of UK SMI publication		

8.3 Oxidase test

Please refer to [UK SMI TP 26: Oxidase test](#).

Neisseria species are oxidase positive (7).

Note: *Kingella* species and *M. catarrhalis* are also oxidase positive and can be misidentified as *Neisseria* species (5,43).

8.4 Matrix-assisted laser desorption/ionisation - time of flight mass spectrometry (MALDI-TOF MS)

MALDI-TOF MS is often used as the primary method for the identification of *Neisseria* species in many diagnostic laboratories. 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 (17).

MALDI-TOF MS has excellent performance for *N. gonorrhoeae* identification (18,61) and may be used as a single diagnostic assay for *N. gonorrhoeae* if reliable species identification score is obtained and the above points are also adhered to.

MALDI-TOF MS is also highly accurate for the identification of *N. meningitidis*, however closely related *Neisseria* species such as *Neisseria polysaccharea* and *Neisseria cinerea* may be misidentified as *N. meningitidis* (17-19). While the identification of non-pathogenic *Neisseria* to species level is generally not required, the misidentification of commensal strains as *N. meningitidis* can have serious health and social consequences.

Confirmation of MALDI-TOF MS results may be required in certain cases, particularly if there are discrepancies in results, if a species identification score is low or not obtained and for medicolegal cases. Confirmation may also be required in low prevalence settings. In such cases, in-house confirmation using conventional or molecular methods should be performed or the isolates can be sent to a STI reference or specialist testing laboratory that offers confirmatory services.

8.5 Further identification

8.5.1 Identification tests and commercial systems

Biochemical, immunological, and preformed enzyme tests offer an alternative approach for identification when MALDI-TOF MS is not available, at least two of which should be used to identify *Neisseria* species. In case of discrepancies in results refer isolates to the appropriate reference or specialist testing laboratory. These tests can also be used to confirm MALDI-TOF MS results if required.

Commercially available kits can also be used. The accuracy of these kits has not been fully determined for species other than *N. gonorrhoeae* and *N. meningitidis* therefore, all results obtained should be interpreted with caution (62).

Laboratories should follow manufacturers' instructions and rapid tests and kits should be validated and shown to be fit for purpose prior to use.

8.5.1.1 Biochemical test

Biochemical tests including commercial kits provide basic biochemical information that can aid in the identification of *Neisseria* species. However, relying solely on these

tests is insufficient for accurate identification of *Neisseria* species. Therefore, these tests are not considered reliable for the primary identification of *Neisseria* species.

Refer to manufacture's guidance or the Manual of Clinical Microbiology for the biochemical properties of *Neisseria* species (7).

8.5.1.2 Immunological and preformed enzyme tests

Currently, there are limited immunological and preformed enzyme detection kits available for the identification of *Neisseria* species. *N. gonorrhoeae* that have a mutation in the proline iminopeptidase gene, and therefore appear negative for this enzyme, have been detected in England and Wales. Kits that only detect the production of aminopeptidases should not be used alone (62,63).

Many of the biochemical kits allow for the combined detection of carbohydrate utilisation and aminopeptidases, but *N. gonorrhoeae* that are proline iminopeptidase negative will give anomalous results with these kits as well and should be confirmed with an immunological reagent.

8.5.1.3 Slide agglutination test

Commercial slide agglutination tests can be used for further characterisation of *N. meningitidis* to serogroup level (7,64). Slide agglutinating sera are for use on cultures only. Heated clinical samples or formalin treated suspensions of cultures should be processed within microbiological safety cabinets to reduce aerosols.

8.5.2 Molecular methods

Molecular methods can serve as alternative identification methods to MALDI-TOF MS or be used for confirmation in cases where MALDI-TOF MS results need validation.

8.5.2.1 Next Generation Sequencing

Next Generation Sequencing technologies that have been largely restricted to reference or specialist testing laboratories are gradually becoming more accessible and cost-effective. Clinical laboratories may implement them for routine identification and diagnostic purposes in the future.

9 Storage

Short term storage – isolates should be kept in a viable state on heated blood (chocolate) agar slopes in 5 - 10% CO₂ at 35 - 37°C.

Long term storage – isolates should be frozen at - 20°C to - 80°C in glycerol based medium or cryo-beads (65).

Note: *N. gonorrhoeae* storage is recommended at - 70°C and below.

10 Reporting

10.1 Infection Specialist

Inform the infection specialist of all confirmed *N. meningitidis* isolates, and of all *Neisseria* species isolated from normally sterile sites, or in cases of invasive infection.

The infection specialist should also be informed if the request bears relevant information, for example:

- cases of meningitis, septicaemia (especially with purpuric rash)
- investigation of *N. meningitidis* outbreak, or of the carrier state

Inform the infection specialist of all confirmed *N. gonorrhoeae* isolates, and of all *Neisseria* species from:

- minors
- cases of sexual assault, rape or abuse
- cases of *N. gonorrhoeae* isolated from normally sterile sites or from invasive infection
- Ceftriaxone resistant *N. gonorrhoeae* isolates from all sites (66).

These are general guidelines. Please follow local protocol for reporting to clinician.

10.2 Presumptive identification

Please note the risk of misdiagnosis with presumptive identification of *Neisseria meningitidis* or *Neisseria gonorrhoeae*. Refer to Section 8: Identification for the full identification of *Neisseria* species.

10.3 Confirmation of identification

In certain cases, confirmation of identification may be required. Refer to Section 8: Identification for identification and confirmation of *Neisseria* species.

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 (35).

10.6 Infection prevention and control team

Inform the infection prevention and control team of confirmed isolates of *N. meningitidis*.

11 Referral to reference or specialist testing laboratories

In case of sending away isolates to reference or specialist testing laboratories for processing, ensure that the specimen is placed in the appropriate package and transported accordingly. Follow local regulations and instructions provided by the reference or specialist testing laboratories for sending isolates.

N. gonorrhoeae – all isolates of *N. gonorrhoeae* from normally sterile sites or from invasive infection and ceftriaxone resistant isolates from all sites need to be sent to the appropriate reference laboratory.

N. meningitidis – all isolates of disease-causing *N. meningitidis* or *N. meningitidis* isolates from a throat swab in a patient being treated with invasive disease need to be sent to the appropriate reference laboratory.

Contact the appropriate reference laboratory (links below) for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission.

[England](#)

[Wales](#)

[Scotland](#)

[Northern Ireland](#)

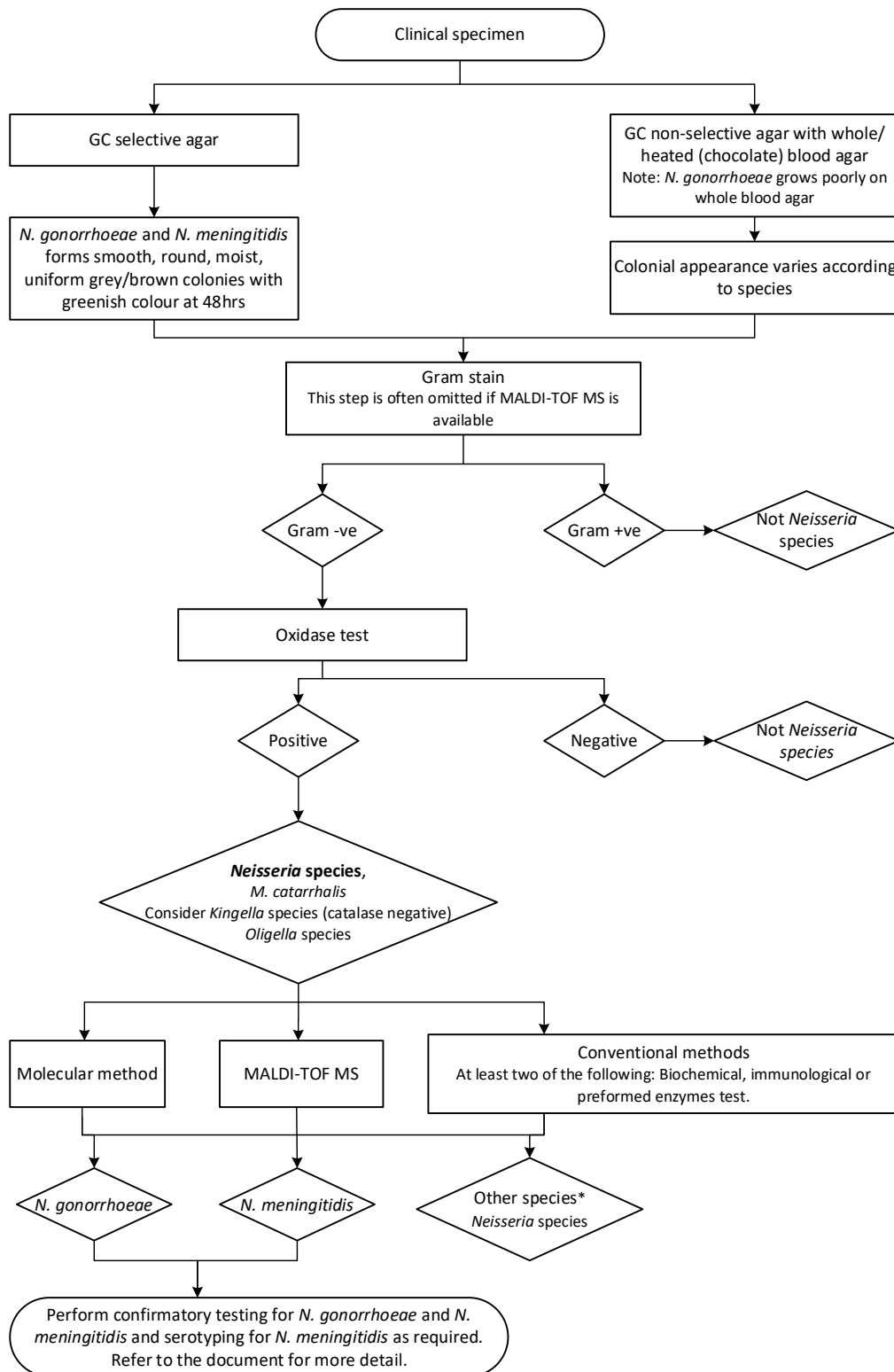
12 Public Health responsibilities of diagnostic laboratories

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 *Neisseria* species



*Report as not *N. gonorrhoeae* or *N. meningitidis*.

The flowchart is for guidance only.

References

An explanation of the reference assessment used is available in the [scientific information section on the UK SMI website](#).

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